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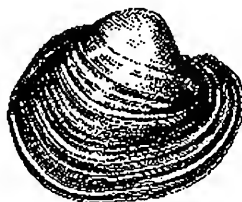
**Value of Mussel Beds to Sport Fisheries**

Final Report, F-80-R

Center for Aquatic Ecology

**Philip B. Moy and Richard E. Sparks**

December 1991



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Project F-80-R

Final Report

Philip B. Moy and Richard E. Sparks

Illinois Natural History Survey  
Forbes Biological Station  
P.O. Box 599  
Havana, Illinois 62644

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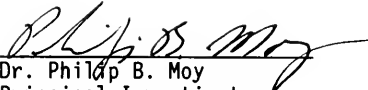
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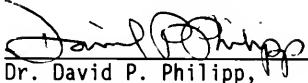
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## ABSTRACT

The larvae, or glochidia, of freshwater mussels are obligate parasites on fish, including sport fish. The host fish provides a means of dispersal and nutrients that enable the glochidia to transform into juvenile mussels. Although some mussels use visual lures (mantle flaps or glochidial packets that look like minnows, worms, or other types of forage) to attract fish, many mussels that are common in the Midwest do not have such obvious means of attracting fish. The purpose of this research was to determine whether one forage fish (fathead minnow) and five common sport fishes (channel catfish, largemouth bass, bluegill, white crappie, and sauger) were more attracted to mussel beds than to other types of substrate, and if they were, to determine the basis for that attraction.

Individual sport fish and schools of fathead minnows were given a choice of two substrates in laboratory tanks. Substrates included mussels and cobbles with naturally occurring attached algae and invertebrates; mussels with cleaned shells; clean cobbles; and bare sand. The mussels were partially buried in sand in a natural position, with their pseudosiphons and the posterior portions of their shells above the sand surface. The cobbles were approximately the same size as the mussels and similarly placed in sand. Each fish was exposed to the same substrate (bare sand or clean cobbles) on both left and right sides of the tanks during a control trial to quantify its side preference. Software running on Apple IIGS computers linked to image digitizing cameras determined the location of the fish every 2 seconds during the day and every 4-8 seconds at night, when the room lights were



automatically dimmed and longer exposure intervals were required by the cameras. A substrate was defined as preferred only if the fish was located more frequently over the side with that substrate than during the control trial.

Fish were located more frequently over mussels and cobbles with attached algae and invertebrates than over clean cobbles or sand. The invertebrates were an obvious attraction that the fish consumed within a few minutes. The preference for mussels and cobbles usually diminished during the 1- to 3-day duration of the trials, a behavior that might be attributed to an initial food reward, followed by a gradual decline in unrewarded searching of the same substrate. However, there was some other attraction associated with the mussels themselves because there was no statistically significant difference ( $P > 0.05$ ) in preference between mussels with cleaned shells and mussels or cobbles with attached algae and invertebrates. When handled, some of the mussels aborted glochidial packets that the fish readily consumed. The aborted packets contained 55-77% protein on a dry weight basis and the embryonic larvae had scarcely any shell material. The protein content dropped to 20% in mature packets, because shell material comprised a greater portion of the total mass. Both immature and mature packets appear to be a nutritious food source that presumably can be digested by the fish--the only glochidia that survive are the ones that attach externally to the gills or fins of the fish. In contrast to the glochidial packets, the feces and pseudofeces produced by the mussels did not appear to be very nutritious for the fathead minnow, a species known to consume detritus; fathead minnows maintained in tanks with mussels lost as much, or more

weight than fish held alone. In predator-prey trials, the minnows were twelve times more vulnerable to sauger when the minnows were over bare substrate than when over cobbles, and consequently they spent twice as much time over the cobbles. The minnows presumably would use mussels similarly as a refuge from predation, although time did not permit testing that hypothesis in additional predation trials. The possibility that mussels release odors that attracted their fish hosts was not investigated, but should be the subject of additional research. During these preference trials, none of the mussels displayed lures that could have visually attracted the fish.

These results suggest that mussels serve as both direct and indirect sources of forage for game fish. The glochidial packets released by the mussels may provide a seasonally abundant food reward for the fishes that disperse the larval mussels. Young game fish may consume invertebrates that colonize mussel shells, or the link may be from invertebrates to small fish, such as fathead minnows, to the piscivores. Small fish also may concentrate in mussel beds to avoid predators or currents. Mussels can serve as solid substrate and refuges for other invertebrates and forage fish because most native mussels continually expose a portion of their shells above the sediment surface, rather than completely burying themselves. Although cobbles can perform the same functions, cobbles can be covered by silt or sand whereas the living mussel actively maintains its position at the sediment surface. The solid structure provided by mussel beds is likely to be most critical as a substrate in alluvial rivers otherwise dominated by shifting deposits of sand or mud.

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## INTRODUCTION

Today habitat degradation in large Midwestern rivers threatens the health of populations of many aquatic organisms. Siltation specifically threatens to cover cobble and gravel substrates which form spawning and feeding areas for riverine fishes. These areas cannot be maintained free of silt without additional human intervention.

Like cobble, mussels provide a firm substrate for attachment of invertebrates (Anderson and Vinikour 1984) and spawning of fishes (Pitlo 1989). Unlike cobble, mussels maintain their position despite sedimentation or scouring and produce feces and pseudofeces that are consumed by other invertebrates (Izvekova and Lvova-Katchanova 1972).

Need for research. Because mussels depend upon fish for completion of their life cycle (Ellis 1929) we know that fish associate with mussels at least during the period of glochidial release. It is not known what effect, if any, elimination of mussel beds through loss of suitable habitat, deteriorating water quality, and overharvest (Fuller 1978) may have on fish.

Mussels have been commercially harvested for various reasons over the last century. In the latter part of the nineteenth century mussels were harvested for food and for the pearls infrequently found in their mantle cavity. Later, in the 1890's and early part of this century mussels were harvested for the pearl button industry. Today

mussels are harvested for their shells which are ground into spheres and implanted in oysters as nuclei for cultured pearls (Fuller 1974).

The vast natural mussel beds in Midwestern United States rivers supported a thriving pearl button industry for many years, though mussel harvests were already beginning to decline in the early 1900's (Eckblad 1986). The decline in populations and the economic importance of mussels fostered the need for research on life history and propagation with the intent to restock mussel beds to support the button industry (Fuller 1974, Coker et al. 1921). The result was a large body of information regarding the life habits of these animals. Subsequent to World War II the advent of plastics resulted in the closing of the pearl button factories and elimination of the industry. Unfortunately this also resulted in elimination of the primary reason for mussel research (Starrett 1971, Fuller 1974).

There have been few investigations of fish substrate preference that have produced conclusive results. Some authors suggest that fish substrate preference is based upon forage organisms associated with the substrate (Rankin 1986), though interstitial space may be important for small fish (Sechnick et al. 1986, DeMarch 1976). Work performed on substrate preference of invertebrates has produced mixed results, but again authors suggest food is influential in substrate selection (Egglishaw 1964), though particle size and interstitial space (Cummins and Lauff 1969), and current velocity and dissolved oxygen are suggested as factors as well (Eriksen 1966).

The majority of information on fish-mussel interactions comes from the mussel literature pertaining to parasitism of fish by mussels (Howard and Anson 1922, Coker et al. 1921), identification of host fish for endangered mussels (Holland-Bartels 1990, Miller et al. 1986, Zale and Neves 1982, Sephton et al. 1980, Stern 1978), use of fish to propagate mussels (Coker et al. 1921, Howard 1914, 1917), and species of fish caught over mussel beds (Wilson and Clark 1912). Other fish - mollusk information pertains to fish consumption of snails or mussels as forage (Bennett and Gibbons 1972, Forbes and Richardson 1908). Some of the early papers speculate on reasons for the presence of fish over mussel beds and cite food or forage as the probable cause. Yet no one has closely examined this relationship to clearly identify whether fish are more attracted to mussels than to non-living substrates.

Parasitism of fish. Larval mussels were originally thought to be parasites infesting the gills of mussels, and were given the name (Glochidium parasiticum). In 1832 Carus proved that these parasites were actually larvae of the mussel itself, though the term glochidium is still used in reference to the larval stage (LeFevre and Curtis 1912). The dependence of mussels on fish for completion of their life cycle was discovered in 1866 when Leydig identified parasites on the gills of fish as glochidia (Ellis 1929). The glochidia of nearly all species of unionid mussels must parasitize a fish for a short time after leaving the female mussel (Clark and Stein 1921) and often display a high degree of host specificity for successful completion of larval development (Howard 1914, 1917, Howard and Anson 1922, Zale and Neves 1982). The larval mussel attaches to the gill lamellae or fins

of the fish and remains there for a period of days or weeks depending on temperature (Davenport and Warmuth 1965, Zale and Neves 1982), mussel species and developmental stage of the glochidium (Howard and Anson 1922). During this period of parasitism the fish provides the young mussel with nutrition for metamorphosis (Arey 1932, Ellis 1929) and with a means of dispersal (Coker et al. 1921, Starrett 1971, Fuller 1974). Encysted larvae metamorphose to the juvenile stage at various rates and apparently do not drop off en masse; glochidia encysted on one fish from one mussel may excyst over a period of two weeks or more (LeFevre and Curtis 1912, Howard 1922).

Many fish are hosts for freshwater mussels. In their 1912 survey of the mussel fauna of the Kankakee River basin Wilson and Clark made seine hauls over mussel beds and recorded the various species of fish they captured. Similarly, Wiles (1975) used seine hauls, dip nets and electrofishing to sample the fish over the mussel beds he studied in Nova Scotia. In their study of lampsilid fish hosts, Zale and Neves (1982) captured several species of fish over mussel beds in Big Moccasin Creek, Virginia. Table 1 provides a compilation of fish captured in the above studies along with fish known to be glochidial hosts.

Table 1. Fish which associate with mussel beds. \* Denotes fish not known to be glochidial hosts which have been captured over mussel beds. Unmarked fish are known mussel hosts. Numbers correspond to forage items, upper and lower case letters refer to citations for association with mussels and diet respectively. See end of table for key.

Species	Diet
<b>Petromyzontidae</b>	
<u>Petromyzon marinus</u> <sup>A</sup>	8 (parasitic) <sup>a,b</sup>
<b>Acipenseridae</b>	
<u>Scaphirhynchus platorhynchus</u> <sup>A</sup>	3-5 <sup>a</sup>
<b>Lepisosteidae</b>	
<u>Lepisosteus platostomus</u> <sup>A</sup>	8 <sup>a,c</sup>
<u>L. spatula</u> <sup>A</sup>	8 <sup>a,c</sup>
<u>L. osseus</u> <sup>A</sup>	8 <sup>a,b</sup>
<b>Amiidae</b>	
<u>Amia calva</u> <sup>A</sup>	8, crayfish <sup>a,b</sup>
<b>Anguillidae</b>	
<u>Anguilla rostrata</u> <sup>A</sup>	3-8, crayfish, snails <sup>b</sup>
<b>Clupeidae</b>	
<u>Alosa chrysochloris</u> <sup>A</sup>	3-8 <sup>c</sup>
<u>A. pseudoharengus</u> <sup>D,F</sup>	1,2, amphipods <sup>b</sup>
<u>Dorosoma cepedianum</u> <sup>A</sup>	2,9 <sup>a,b</sup>
<b>Salmonidae</b>	
<u>Oncorhynchus mykiss</u> <sup>A</sup>	2-8, snails, crustaceans <sup>a,b</sup>
<u>O. nerka</u> <sup>A</sup>	2 <sup>b</sup>
<u>O. trutta</u> <sup>A</sup>	3-8, crustaceans <sup>a,b</sup>
<u>O. tshawytscha</u> <sup>A</sup>	3-7, crustaceans (in fresh water) <sup>b</sup>
<u>Salvelinus fontinalis</u> <sup>A</sup>	3-8, crustaceans <sup>a,b</sup>



Table 1 cont'.

## Esocidae

Esox americanus \*B  
E. lucius B,E

6,8 amphipods, isopods<sup>C</sup>  
 6,8<sup>C</sup>

## Cyprinidae

Campostoma anomalum \*C  
Carassius auratus A  
Chrosomus eos \*B  
Cyprinus carpio A  
Ericymba buccata \*B  
Hybopsis amblops \*B,C  
Nocomis biguttatus \*B  
N. micropogon \*C  
Notemigonus crysoleucas A,B,D  
Notropis ardens A  
N. blennius \*B  
N. coecogenis \*C  
N. cornutus \*B,C,D  
N. galacturus \*C  
N. heterodon \*B  
N. heterolepis \*D  
N. leuciodus \*C  
N. rubellus \*C  
N. spilopterus \*B  
N. teloscopus \*C  
N. umbratilis \*B  
N. whipplei \*B  
Phenacobius mirabilis \*B  
Pimephales notatus \*B,C  
P. promelas \*B  
P. vigilax \*B  
Rhinichthys atratulus \*C  
R. osculus A  
Richardsonius egregius A  
Semotilus atromaculatus B

5,9<sup>d</sup>  
 1-7<sup>a</sup>  
 1-7<sup>b</sup>  
 1-7,9<sup>a</sup>  
 2,3,5,9<sup>c</sup>  
 6,7<sup>a</sup>,<sup>c</sup>  
 1,4-6<sup>c</sup>  
 1,2-5,8, snails, crayfish<sup>b</sup>  
 1,2,6,7,9, molluscs<sup>C</sup>  
 1, "animal material"<sup>a</sup>  
 1,2,6<sup>C</sup>  
 1-5,7-9<sup>d</sup>  
 1,2,5<sup>C</sup>  
 1-7<sup>a</sup>  
 1,3,5-7,9<sup>d</sup>  
 1-5,7<sup>d</sup>  
 Unknown<sup>a</sup>  
 1,6,8<sup>C</sup>  
 3-5,9<sup>c,d</sup>  
 1-6,9<sup>c,d</sup>  
 1,5,7,9<sup>c,d</sup>  
 1,2-5,7,9<sup>d</sup>  
 1,4,5<sup>b</sup>  
 1-5<sup>e</sup>  
 1-5,8,9<sup>c,d</sup>

## Catostomidae

Catostomus commersoni B,C,D,G  
C. tahoensis A  
Carpionotus velifer A  
Erimyzon sucetta B  
Hypentelium nigricans B,C,G  
Moxostoma macrolepidotum A  
M. duquesnei \*B,C

3-5, molluscs, crustaceans<sup>C</sup>  
 2,3-5,9, Sphaerium, Lymnea<sup>C</sup>  
 "benthic invertebrates"<sup>a</sup>  
 3,5,9, mussels<sup>C</sup>  
 2-5,9, Sphaerium, Snails<sup>C</sup>  
 6,9, molluscs<sup>d</sup>

Table 1 cont'.

Ictaluridae

<u>Ictalurus melas</u> <sup>A</sup>	1,3-8, mussels, snails, crustaceans <sup>C</sup>
<u>I. natalis</u> <sup>C</sup>	1-8 <sup>C</sup>
<u>I. nebulosus</u> <sup>A</sup>	1-8, mussels, snails <sup>C</sup>
<u>I. punctatus</u> <sup>A</sup>	1-8, snails, <u>Anodonta</u> <sup>C</sup>
<u>Noturus gyrinus</u> <sup>B,E</sup>	2-5, amphipods, isopods <sup>C</sup>
<u>Pylodictus olivarius</u> <sup>A</sup>	8, crustaceans <sup>a,c</sup>

Cyprinodontidae

<u>Fundulus diaphanus</u> <sup>B,D</sup>	2,6,7, snails, amphipods, odonates <sup>C</sup>
<u>F. dispar</u> * <sup>B</sup>	1,2,6,7, snails <sup>a,c</sup>
<u>F. zebrinus</u> <sup>A</sup>	

Poeciliidae

<u>Gambusia affinis</u> <sup>A</sup>	2-6 <sup>a</sup>
--------------------------------------	------------------

Atherinidae

<u>Labidesthes sicculus</u> * <sup>B</sup>	2,5,7 <sup>C</sup>
--	--------------------

Gasterosteidae

<u>Apeltes quadracus</u> <sup>D</sup>	1,2 <sup>b</sup>
<u>Culea inconstans</u> <sup>A</sup>	1-5, snails <sup>a,b</sup>
<u>Gasterosteus aculeatus</u> <sup>D</sup>	3-8
<u>Pungitius pungitius</u> <sup>D</sup>	2-6 <sup>a,b</sup>

Percichthyidae

<u>Morone americana</u> <sup>D</sup>	2-5,8 <sup>b</sup>
<u>M. chrysops</u> <sup>A</sup>	2-8 <sup>a,b</sup>

Centrarchidae

<u>Ambloplites rupestris</u> <sup>B,C</sup>	2-8, crustaceans <sup>a,b</sup>
<u>Lepomis cyaneellus</u> <sup>A</sup>	2-8 <sup>b</sup>
<u>L. gibbosus</u> <sup>A,B</sup>	2-8, molluscs <sup>a,b,c</sup>
<u>L. gulosus</u> <sup>A,B,E,G</sup>	2-8 <sup>a,c</sup>
<u>L. humilis</u> <sup>A</sup>	2-8 <sup>a</sup>
<u>L. macrochirus</u> <sup>A,B</sup>	1-8, molluscs <sup>b,c</sup>
<u>L. megalotis</u> <sup>A</sup>	2-8 <sup>b</sup>
<u>Micropterus dolomieu</u> <sup>B,C,E,G</sup>	2,3,6-8, crustaceans <sup>a,c</sup>
<u>M. salmoides</u> <sup>B,C,E,H</sup>	2,3,6-8, crayfish <sup>a,c</sup>
<u>Pomoxis annularis</u> <sup>B,E</sup>	3-6,8, crustaceans <sup>a,b,c</sup>
<u>P. nigromaculatus</u> <sup>A</sup>	2-8 <sup>b</sup>

Table 1 cont'.

Percidae

<u>Etheostoma blennoides</u> * <sup>C</sup>	2-5, benthos <sup>a,b</sup>
<u>E. caeruleum</u> * <sup>B</sup>	3-5 <sup>a</sup>
<u>E. exile</u> <sup>B</sup>	2-5, amphipods, snails <sup>a,b</sup>
<u>E. flabellare</u> <sup>C</sup>	3,4,6, coleoptera larvae <sup>b</sup>
<u>E. microperca</u> * <sup>B</sup>	2,3,5, amphipods <sup>a,b,c</sup>
<u>E. nigrum</u> <sup>B</sup>	2,3,5 <sup>b</sup>
<u>E. rufilineatum</u> <sup>C</sup>	1,2, drift <sup>f</sup>
<u>E. simoterum</u> * <sup>C</sup>	benthos <sup>f</sup>
<u>Perca flavescens</u> <sup>A,B,D,E,I</sup>	3-5,8 <sup>b,c</sup>
<u>Percina caprodes</u> * <sup>B</sup>	2,5,6, molluscs <sup>a,c</sup>
<u>P. phoxocephala</u> * <sup>B</sup>	3,5,6, odonates <sup>a,c</sup>
<u>P. maculata</u> * <sup>B</sup>	3,5,6, odonates <sup>a,c</sup>
<u>Stizostedion canadense</u> <sup>A</sup>	2-6,8, crayfish <sup>a,b</sup>
<u>S. vitreum vitreum</u> <sup>A</sup>	2-8, crayfish <sup>a,c</sup>

Sciaenidae

<u>Aplodinotus grunniens</u> <sup>A</sup>	2-6,8, mussels, snails, crustaceans <sup>C</sup>
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Cottidae

<u>Cottus bairdi</u> <sup>A,B</sup>	3-5, crayfish <sup>a,c</sup>
<u>C. caroliniae</u> <sup>B,C</sup>	6,8, crustaceans <sup>a,c</sup>

Key to forage items: 1 - algae; 2 - zooplankton; 3 - Ephemeroptera nymphs; 4 - Trichoptera larvae; 5 - Diptera larvae; 6 - adult aquatic insects; 7 - adult terrestrial insects; 8 - fish; 9 - bottom ooze (diatoms).

Key to fish species citations: A - Fuller (1974); B - Wilson and Clark (1912); C - Zale and Neves (1982); D - Wiles (1975); E - Coker et al. (1921); F - Davenport and Warmuth (1965); G - Howard and Anson (1922); H - Arey (1932); I - Tedla and Fernando (1969).

Key to diet citations: a - Smith (1979); b - Scott and Crossman (1973); c - Forbes and Richardson (1908); d - Starrett (1950); e - Segler (1963); f - Zale and Neves (1982).

Mussels as forage. Most of the fish species, listed in Table 1 that associate with mussel beds do not consume mussels as forage and many of these species are not known to be glochidial hosts. Minnows and darters present over mussel beds feed on invertebrates, vegetation and "bottom ooze" (diatoms). Some predacious drift feeders will feed on glochidia but there is no indication of selection for glochidia (Zale and Neves 1982). Zooplankton comprise the initial food of larval sport fish. As the young fish grow, insect nymphs, larvae and small fish comprise an increasing portion of their diet (Gerking 1962, Ney 1978) and as larger adults, sport fish will consume minnows, darters and young of their own and other fish species (Forbes and Richardson 1908, Smith 1979, Scott and Crossman 1973).

A few fish species, notably the freshwater drum (Aplodinotus grunniens), channel catfish (Ictalurus punctatus), blue catfish (I. furcatus) and the redear (Lepomis microlophus) regularly consume mussels as forage (Forbes and Richardson 1908, 1920, Howard 1913, Wilbur 1969); other species such as the largemouth bass (Micropterus salmoides) may occasionally consume mussels (Bennett and Gibbons 1972). Yet most fish known to be hosts for mussels are not particularly noted for their consumption of mussels. Fuller (1974) suggests that a mutualistic relationship exists between fish and mussels because fish which have been infected with glochidia gain resistance to parasitic copepods. Howard (1913) suggests a relationship similar to that between plants and pollinating insects may exist for fish and mussels and that mussel beds are attractive to fishes because of the associated invertebrate life in the vicinity.

Coker et al. (1921) also speculated that food may be the "clue to unraveling the mystery" as to why fish are found near mussel beds.

Mantle flap lures. The existence of lures used by mussels to attract potential host fish further suggests that glochidial hosts are foraging in areas inhabited by mussels. Several species of Lampsilis possess mantle flaps which mimic the form and movements of a small fish (Kraemer 1970, Harman 1970, Wickler 1978, Welsh 1969) and may serve to attract their piscivorous, sight-feeding hosts. Lampsilisteres displays the marsupium (the gills containing the glochidia) and the mantle flaps when the glochidia are ready to parasitize a host fish (Appendix A). Similarly, during the period of glochidial release, Villosa nubilosa emerges completely out of the substrate, gapes the valves and fully extends its foot out of the shell (Zale and Neves 1982). Smallmouth bass (M. dolomieu), largemouth bass, bluegill (Lepomis macrochirus), rock bass (Ambloplites rupestris), white crappie (Pomoxis annularis), yellow perch (Perca flavescens), walleye (Stizostedion vitreum vitreum) and sauger (S. canadense) are among the known hosts for these mussels (Clarke 1981, Mathiak 1979, Fuller 1974, Waller et al. 1985)--all are sight-feeding predators.

Glochidial conglutinates. An additional example of a lure to attract foraging fish is found in the Arkansas fanshell (Cyprogenia aberti) which forms red, worm-like glochidial conglutinates which protrude from the shell opening (Chamberlain 1934). The conglutinates are composed of thousands of glochidia held together in a mucous matrix

with mature glochidia on the outside and immature glochidia on the inside. Chamberlain observed these worm-like projections were readily consumed by fish. When the fish picks up the packet, mature glochidia on the surface of the packet break free in the buccal cavity of the host, pass into the gill chamber and attach to gill filaments. The remainder of the packet is consumed by the fish. Chamberlain speculated that the conglomerates resembled tubificids or other bottom-living worms; chironomid larvae are known to associate with mussel shells (Beedham 1970, pers. obs.) and would likely have a similar appearance. Often when mussels are disturbed, as occurs with collecting, they expel glochidial conglomerates. These packets are subcylindrical or flattened in cross section with pointed or blunt ends and are readily consumed by bluegill (pers. obs.).

Other invertebrates and mussels. Non-bivalve macroinvertebrates, as indicated above, are food for many fishes and are also found in association with mussels. These invertebrates find food and firm substrate for attachment in mussel beds. Trichopteran and chironomid larvae have been found on mussel shells (Anderson and Vinikour 1984 and Beedham 1970) and Driscoll and Brandon (1973) in their study of fossil sediments found a greater abundance and diversity of suspension feeding invertebrates on bottom sediments which have higher concentrations of dead shells; material which may serve as attachment sites. Types of invertebrates which have been found in association with mussels appear in Table 2.

Table 2. Invertebrates found in association with mussels. Lower case letters refer to references as follows: a - Coker et al. (1921), b - Sephton et al. (1980), c - Anderson and Vinikour (1984) and d - Beedham (1970).

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Turbellaria <sup>a</sup>	Insecta
Bryozoa <sup>a</sup>	Plecoptera <sup>a</sup>
Mollusca	Ephemeroptera
Gastropoda	<u>Heptagenia</u> <sup>a</sup>
Viviparidae	Odonata
<u>Vivipara</u> <sup>a</sup>	<u>Gomphus</u> <sup>a</sup>
Pleuroceridae	<u>Argia</u> <sup>a</sup>
<u>Pleurocera</u> <sup>a</sup>	<u>Neurocordulia</u> <sup>a</sup>
Pelecypoda	Trichoptera
Sphaeriidae	Hydropsychidae
<u>Musculium</u> <sup>a</sup>	<u>Hydropsyche</u> <sup>a</sup>
Annelida	Leptoceridae
Oligochaeta	<u>Oecetis</u> <sup>c</sup>
<u>Enchytraeus</u> <sup>b</sup>	Coleoptera <sup>a</sup>
Hirudinea	Diptera
<u>Placobdella</u> <sup>a</sup>	Chironomidae
Crustacea	Chironominae
Decapoda	<u>Harnischia</u> <sup>b</sup>
<u>Cambarus</u> <sup>a</sup>	<u>Tanytarsus</u> <sup>b</sup>
	<u>Micropsectra</u> <sup>b</sup>
	<u>Dicrotendipes</u> <sup>b</sup>
	<u>Polypedilum</u> <sup>b</sup>
	Tanypodinae
	<u>Procladius</u> <sup>b</sup>
	Orthocladiinae <sup>b</sup>
	<u>Metriocnemus</u> <sup>d</sup>

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Sephton et al. (1980) found a greater numerical abundance of invertebrate organisms in areas inhabited by mussels than in areas devoid of mussels in a New Brunswick reservoir and theorized the increase was in response to an increased food source. Sephton and his coworkers identified a positive association between Procladius, an omnivorous chironomid, and mussels. These workers suggest the positive association could be an indirect response of a predator (Procladius) to higher prey densities in the form of detritus feeders in the vicinity of the bivalves.

Like cobble, there is an organic coating on mussel shells, composed of bacteria, attached algae and bryozoans, but mussels also produce feces and pseudofeces which can provide forage for aquatic insect nymphs and larvae occupying the "scraper" or "collector" functional groups outlined by Merritt and Cummins (1984) and Wetzel (1983). These groups include Insecta orders Ephemeroptera, Trichoptera, and Diptera which would be found in lotic habitats. The detritivorous "collectors" in particular could feed upon mussel feces and pseudofeces and the protein in these materials may enhance the forage value of sediments and detritus in the region of the mussel bed.

Feces as forage. Detritivorous invertebrates consume their own feces as well as that of other species (Hynes 1970). The isopod Asellus aquaticus benefits from the consumption of grass carp (Ctenopharygodon idella) feces (Petridis 1990). Grass carp consume macrophytes and break down the material into smaller particles the



isopod can more easily digest. It is reasonable to suppose that this isopod could benefit from the consumption of mussel pseudofeces as well since fecal matter produced by filter feeders may be further processed by "collector" organisms in the stream (McCullough et al. 1979). Bacteria are relatively sparse on detritus until it has passed through the digestive tract of an invertebrate (Hargrave 1976). Taylor and Roff (1984) found food quality of detritus was five times greater in downstream reaches than in the head waters due to bacterial colonization of the detritus.

Pseudofeces. Mussels are not selective in their ingestion of particles; all particles small enough to be drawn into the incurrent siphon are agglutinated into mucous strings. Mussels consume decaying organic matter and animal plankters (Fuller 1974, Burky 1983) but may also consume phytoplankton (Carver and Mallet 1990, Shpigel and Fridman 1990). As they feed, mussels filter several liters of water daily and in doing so sediment planktonic matter (Leff et al. 1990). When the concentration of food material exceeds that required for maintenance or growth, some of the food is egested, prior to digestion, as pseudofeces (Winter 1978).

Pseudofeces as forage. Izvekova and Lvova-Katchanova (1972) demonstrated that the feces and pseudofeces produced by Dreissena polymorpha provide a more nutritious forage for chironomids than the same material which has not been agglutinated by mussels and suggest that the mucopolysaccharide coating on the agglutinated matter protects the contents from the effects of leaching thus making the

contents more nutritious than uncoated detritus. The excretory and feeding by-products deposited by the mussels are more densely colonized by bacteria than unfiltered material which further enhances the nutritional value of these products for detritivores since bacteria are a major nutritional component of the detritivore diet (Brinkhurst 1974).

Much recent work has been done on filtration rates of the zebra mussel Dreissena polymorpha and Asiatic clam Corbicula fluminea but there are few recent studies on the filtering rate of unionid mussels. The zebra mussel belongs to the superfamily Dreissenaceae and the Asiatic clam belongs to the superfamily Corbiculaceae. The mass of material sedimented by these mussels per unit area is greater than that of mussels in the superfamily Unionaceae because the Dreissenaceae and Corbiculaceae can occur at higher densities, but their ecological role as filter feeders is similar.

Wisniewski (1990) measured a clearance rate of  $4.2 \times 10^{-6}$  -  $5.0 \times 10^{-6}$  pounds/hour for a 0.86 inch specimen of D. polymorpha and a filtration rate of  $5.3 \times 10^{-4}$  -  $7.58 \times 10^{-2}$  gal/mussel/h. He estimates a total of 0.017 lbs dry weight/ft<sup>2</sup>/day is sedimented by these mussels in lake Parteczyny. Izvekova and Lvova-Katchanova (1972) measured a filtration rate of  $2.42 \times 10^{-5}$  gal/h/lb for D. polymorpha and estimated that the total population of zebra mussels in Uchinskoye reservoir sedimented 53.8 tons of dry matter/day. Reeders and Bij de Vaate (1990) estimate a filtration rate of 0.013-0.015 gal/mussel/hour.

Kryger and Riisgard (1988) find that the filtration rates of freshwater species are two to eight times lower than marine bivalves of comparable size. There is a decrease in the filtration rate with an increase in size. For example, Carver and Mallet (1990) measured a filtration rate of  $6.98 \times 10^{-4}$  -  $1.45 \times 10^{-3}$  gal/hour/lb dry weight for Mytilus edulis while Widdows et al. (1990) report filtration rates for the same species ranging from  $8.9 \times 10^{-4}$  gal/hour/lb for a  $1.54 \times 10^{-3}$  lb mussel and  $3.9 \times 10^{-3}$  gal/hour/lb for a  $4 \times 10^{-4}$  lb mussel. Kryger and Riisgard report that feeding rates of undisturbed freshwater bivalves can be four or more times higher than disturbed bivalves; for this reason they suggest that filtration rates may actually be much higher than has been previously reported. Data on the filtering rates of marine and freshwater mussels are summarized in Appendix C.

Sedimentation of organic matter. Mussel beds may also cause planktonic material to settle by reducing current velocity through friction which disrupts the laminar flow of the water. The friction is caused by the rough surface of mussel beds, and as the current slows, planktonic material settles out of the water column (Holloway 1990). Haven and Morales-Alamo (1966) examined the biodeposition rate of oysters and estimated that seston filtered by the mussels is deposited as feces and pseudofeces about seven times faster than would occur by gravity. Similarly these authors cite Lund (1957) as calculating that oysters covering 1 acre of bottom could deposit 8.35 tons (dry weight) of fecal material in eleven days, equivalent to  $12.86 \text{ lb/ft}^2/\text{yr}$ .

Mussels process suspended organic matter and in this way make the material more available to detritivorous species. Obviously mussels cannot sediment more than what is available in the water column. Still, from this information one can appreciate the potential contribution to the detritus from planktonic material sedimented by mussels in the form of feces and pseudofeces. Bacteria comprise the dominant protein source for detritivorous fishes (Brinkhurst 1974). It is possible that in addition to providing other invertebrates with firm substrate and forage in the form of feces or pseudofeces, freshwater mussels may provide detritivorous fish with an enhanced food resource as well.

Habitat permanence. The low production to biomass ratio (P:B) characteristic of unionid mussels means that a large population of long-lived adults is required to propagate the species. For the Thames river, Negus (1966) suggests a P:B ratio of 1:6 for mussels. Outside of man, adult mussels have few natural predators. The slow degradation of dead shells and long lives of adult mussels combine to make mussel beds a stable habitat which may allow for greater species diversity of other invertebrates which colonize mussel beds (Hargeby 1990)

Interstitial habitat. The interstitial space is important habitat for fish forage organisms (Cummins and Lauff 1969) and may be important for small fishes as well. Cobble substrate forms a temporally unstable refuge for small invertebrates in streams (DeMarch 1976). As silt seasonally fills the interstices the animals are

eliminated from the habitat. Boulder (10 inches) substrate forms a physically more stable habitat because the particles do not move downstream and the interstices, which may be occupied by small invertebrates, crayfish, turtles or small fish, are not as readily filled by siltation.

Immature insects appear to select substrate on the basis of particle size, or more precisely, interstitial space. When Cummins and Lauff (1969) offered invertebrates various sizes of particles associated with and not associated with silt, Caenis latipennis and Perlesta placida selected the interstices of coarse sediments. These authors state that substrate particle size and food supply are the primary macrodistributional influences on invertebrates. Egglisshaw (1964) found a significant correlation between number of invertebrates and plant detritus in riffles and suggested that the animals were associating with their food, rather than accumulating there solely for physical reasons, though Eriksen (1966) asserts that physical conditions such as water currents and oxygen are influential forces in the selection of crevice habitats.

In laboratory studies with smallmouth bass, Sechnick et al. (1986) found substrate was important only when the fish could get into the interstices, while Rankin (1986) found that smallmouth bass in a natural stream select specific substrate types and suggests this is a response to prey distribution.

Substrate heterogeneity. Woodin (1978) suggests that substrate heterogeneity in the form of physical structure such as rock or cobble or biogenic structures such as the tubes built by polychaete worms can form refugia for benthic organisms. She cites the tubes of Diopatra cuprea, a marine polychaete, as an example of a biogenic refuge, one which is capable of renewing itself after destruction by a storm, a characteristic not possessed by refugia of physical origin.

Mussel beds may perform a similar function in freshwater. Live mussels can maintain their position during both flood scour and silt deposition (Kranz 1974) and in doing so provide case-building insect larvae a solid place for attachment (Anderson and Vinikour 1984). Similarly the mussel itself could provide a current break for small benthic organisms (Hynes 1970). In a dense mussel bed the accumulation of dead shell material could provide attachment sites and interstices for non-bivalve invertebrates and spawning sites for lithophilous fishes (Pitlo 1989, Balon 1974).

Bioturbation. Since mussels are living organisms they have the ability to adjust to minor perturbations in the environment (Kranz 1974). Through their repositioning movements or other taxes mussels mix and turn the upper few centimeters of sediment as a plow turns a field. Through their movements, mussels held in an indoor tank with sand overlying gravel soon combine the two substrates into a homogeneous mixture (pers. obs.). In a mussel bed this action could bring shallowly-buried firm substrates to the surface creating attachment sites for other invertebrates and juvenile mussels

(Driscoll and Brandon 1973, Isley 1911) and help aerate the surface sediments for nitrogen and phosphorus cycles (Keeney 1973).

## SUMMARY OF LITERATURE

Habitat degradation threatens many species in Midwest rivers. In many cases maintenance of the habitat by human intervention carries prohibitive costs or is simply impractical. Mussel beds are a substrate suitable for foraging and spawning of fishes and can maintain themselves if not subjected to additional adverse human influences.

Larval mussels complete metamorphosis and become dispersed through parasitism of a host fish. The question arises as to why the host fish occur in proximity to mussels at the time of glochidial release. The above discussion and references suggest that, as Coker and Howard suspected 75 years ago, forage occurring in mussel beds may attract the fish which are hosts for the mussels.

In general glochidial hosts do not consume mussels as forage, but small cyprinids and percids which serve as forage for piscivorous fish do occur over mussel beds, probably in response to increased densities of non-bivalve invertebrate forage organisms attached to and feeding among the mussels. The mixture of mucus, bacteria, and organic particles found in mussel feces and pseudofeces provides nutritious forage for detritivorous invertebrates and is consumed by omnivorous fishes such as the fathead minnow (Pimephales promelas) as well (pers. obs.).



It may be that the fish are responding to an area of increased forage rather than to the mussels themselves. Minnows, darters and young of larger species could find refuge from current or predators among the live and dead shells in mussel beds. These small fishes, together with non-bivalve macroinvertebrates might attract and provide forage for larger predacious fishes.

The existing literature does not provide definitive answers to questions as to why fish occur over mussel beds, whether the fish benefit from this association, or whether the fish prefer mussels to other substrates. This study uses a laboratory approach to examine fish preference for mussels, seeks to identify what factors might attract fish to mussel beds and suggests how fish might benefit from their association with mussels.

## RESEARCH QUESTIONS

- I. Do fish prefer mussels over other substrates?
- II. Is fish response to mussels based on the same factors as fish response to inorganic substrates?
  - A. Is the response based on structure?
    - 1. Do the fish merely prefer to be over heterogeneous substrate?
    - 2. Can cobbles and mussels offer a refuge from predation?
  - B. Is the response based on forage?
    - a. Do the fish respond to invertebrates encrusting mussel shells and cobbles?
    - b. Can fish utilize mussel feces and pseudofeces?
    - c. What is the nutritional content of glochidial packets consumed by fish?

## APPROACH

This study used three approaches in a laboratory setting to examine fish - mussel trophic relationships. The first was a substrate preference study, the second a predator-prey study and the third was a feeding study.

Substrate preference trials determined whether the fish spent more time over mussels than over other types of substrate (cobble and sand) and tested two hypotheses: (1) fish are attracted to the algae and small invertebrates attached to the mussel shells (the forage hypothesis), and (2) fish are attracted by the physical structure provided by the mussel shells (the structure hypothesis). The mussels were partially buried in sand in a natural position, with their pseudosiphons and the posterior portions of their shells above the sand surface. The cobble were approximately the same size as the mussels and similarly placed in sand. Individual sport fish and individuals and schools of fathead minnows were given a choice of two substrates at a time in laboratory tanks.

The forage hypothesis was tested by offering a choice between substrates colonized with attached algae and invertebrates and uncolonized substrates without attached organisms. The colonized substrates were cobble and mussels from streams. Uncolonized substrates were mussels whose shells were scrubbed clean and cobble obtained from roadsides or a quarry. The structure hypothesis was tested by giving fish choices between bare sand and uncolonized cobble

or mussels. To check whether the shells alone were the attraction, rather than the living mussels, three species of fish (bluegill, white crappie, and juvenile channel catfish) were given a choice of empty mussel shells, as well as cobbles and live mussels. The empty shells were still joined in pairs by the elastic hinge ligaments, so they retained the external size and shape of the live mussels and were placed in the preference tanks in the same orientation as the live mussels. Empty shells were stored dry until placed in the preference tanks, and consequently there were no living organisms attached to them.

The predator-prey trials measured the rate at which sauger captured fathead minnows over bare sand versus uncolonized cobbles. In each of six trials five fathead minnows were introduced on two consecutive days to a substrate preference tank containing a single sauger.

The feeding studies stemmed from an observation during the preference trials of fathead minnows consuming mussel feces and a second observation of fish in the holding tanks eagerly consuming packets of glochidial larvae released by mussels. In the feeding trials, fish were held in aquaria alone or with mussels. The only food source provided during the eight two-week trials was from creek water continuously pumped into the aquaria. In every trial but one, the creek water was strained through a 0.01-inch mesh screen to remove most vegetation and invertebrates that were large enough for the fish to consume directly. The screens did not remove small particles that mussels are capable of filtering from the water. Thus the feces from the mussels were the

major source of food, and the utilization and nutritional value could be judged by comparing the weight change of fish held with mussels to fish held alone. In addition, the protein content of glochidial packets was measured to determine their potential nutritional value to fish.

## PREFERENCE STUDY

### METHODS and MATERIALS

Prior to the substrate preference trials we held the fish and mussels in separate systems. We kept fish indoors to acclimate them to confinement in tanks and to rid them of pathogenic agents such as monogenetic trematodes or Ichthyophthirius. We offered the fish live and formulated diets at a maintenance ration ad libitum while in the holding systems.

We held mussels in outdoor tanks or a nearby stream so that the shells would become colonized by non-bivalve invertebrates. We fertilized the outdoor tanks with F/2 algae food from Fritz Aquaculture Supply to encourage algal production and frequently added creek water to provide food for the mussels.

Test chambers. The indoor experimental units offered a choice of two substrates, one placed on either side of the tank. We used two sizes of tanks. A set of six small tanks, 24 inches across the front by 48 inches on a side by 12 inches deep, and another set of three large tanks, 48 inches on a side by 12 inches deep. Baffles on the sides of each tank provided space for motor driven paddles which produced a current for maintenance of the mussels (Figure 1). Water circulated through the system via ports for water passage at both ends of the baffles. Paddles for each tank were driven by a 1/15 hp gear

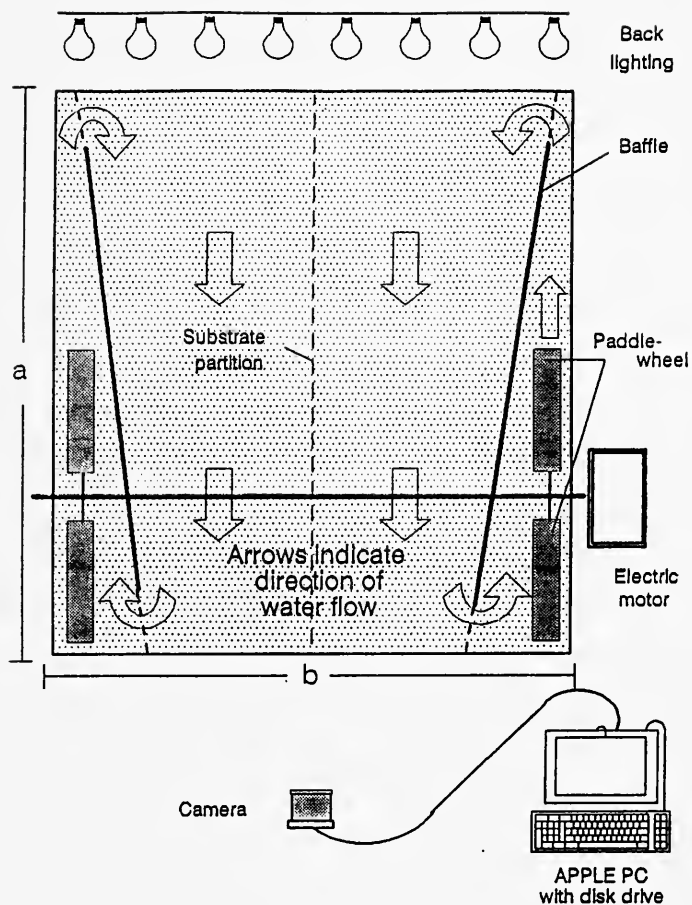


Figure 1. Top view of experimental tank and associated apparatus. a) 48 inches in all tanks. b) 24 inches in small tanks, 48 inches in large tanks.

motor. The motors were connected to individual speed control switches so that uniform paddle velocity could be obtained in all tanks.

Water Quality. Temperature in the experimental system was controlled by room temperature, addition of well water, and aquarium heaters. Water in each tank was pumped through a charcoal/zeolite canister filter to remove ammonia. The rotating paddles which created current for the mussels also aerated the water. Temperature and dissolved oxygen were measured daily, and ammonia and pH were monitored weekly in the holding and experimental units to ensure adequate water quality existed in all tanks. Automatic timers controlled the photoperiod and a partition eliminated entry of sunlight when the building door was opened; there were no windows.

Fish Species. We used adult bluegill, sauger, white crappie, and fathead minnows; one-plus year old channel catfish, and young-of-the-year (YOY) largemouth bass, channel catfish and walleye; and tested each species individually. We used the small tanks with bluegill and one-plus year old channel catfish, and used the wider tanks with sauger, fathead minnow and all YOY fish; white crappie were tested in both sizes of tanks.

We used single individuals of the large species and groups of ten fish with the small or young fish. Gorman (1988) found minnows in tanks formed cohesive schools when six or more individuals of a species were placed together. With the exception of bluegill and one-plus year old channel catfish there were six replicates of each



species. Two bluegill died from a parasitic infection; one catfish escaped. When we used the small tanks, six fish were tested at once. The large tanks took up more space so only three units were available at a time.

Acclimation. The fish were allowed to acclimate in the experimental tank for at least one day and were not fed during data collection. Data collection began after the acclimation period.

Control trials. A trial with the same substrate on both sides of the tank served as a control to determine fish preference for one side of the tank over another. Scrubbed mussels were the control substrate for bluegill, one-plus channel catfish and white crappie in the small tanks; sand was the control substrate for all other fish. The number of times the fish were located over each variable substrate was compared to the control substrate for each species. Table 3 lists the variable and control substrates used with each species.

Variable Substrates. Live mussels colonized with periphyton and perizoon, rocks and live mussels which had been scrubbed clean, and sand were used in all trials. Rocks with perizoon were used with YOY fish and fathead minnows in the large tanks and clean, dead shells were used with bluegill, one-plus channel catfish and white crappie in the small tanks. Two to three inches of sand covered the bottom of the preference tanks. Substrate variables were placed on or in the sand and the sequence of variable presentation was randomized. We used several mussel species as the colonized or clean mussel substrate

Table 3. Variable and control substrates used with each species. Variable substrates are: a - colonized mussels, b - scrubbed mussels, c - sand, d - rock, e - colonized rock, f - dead shells.

Species	Control Substrate	Variable Substrates	Tank Size*
Bluegill	Scrubbed mussels	a,c,d,f	Small
White crappie	Scrubbed mussels	a,c,d,f	Small
" "	Sand	a,b,d	Large
Channel catfish	Scrubbed mussels	a,c,d,f	Small
Fathead minnow	Sand	a,b,d	Large
" "	Sand	a,b,d,e	Large
Sauger	Sand	a,b,d	Large
YOY Walleye	Sand	a,b,d,e	Large
YOY Channel catfish	Sand	a,b,d,e	Large
YOY Largemouth bass	Sand	a,b,d,e	Large

\*) Small - 24 inch wide tanks; large - 48 inch wide tanks.  
YOY) Young-of-the-year fish.

during the preference trials depending on what was on hand at the time. Table 4 lists the mussel species used in the preference trials. Mussels were restricted to one side of the tank by a center barrier in the substrate which did not restrict fish movement (Figure 2).

Data collection. Fish position data was collected with an image digitizing MicronEye camera linked to an Apple II GS computer. Back lighting of the tanks silhouetted the fish and allowed the camera to monitor the position of the fish. The tanks were viewed end-on as in Figure 2. Nocturnal readings were achieved by using a low light level with red light bulbs which produce primarily red and infrared light. The camera is sensitive to red and infrared wavelengths. Fish are relatively insensitive to these wavelengths when acclimated to low light conditions (Brett 1957).

A software program (FishWatcher), developed by Robert F. Illyes, tracked the position of the center of the fish mass (a single fish or a school) and noted whether the center was over the right or left side of the tank (control or variable substrate). The position data were processed and recorded on a disk. At the end of data collection the percent of time the fish spent on the variable side of the tank was determined for statistical analysis.

During a substrate trial data were collected for a minimum of one day and a maximum of three days. Upon termination of a given trial a new substrate was placed in the tank, and data collection began

Table 4. Mussel species used as substrate in the preference trials. Species at the top were used more often than those at the bottom of the list.

Common Name	Species
Giant Floater	<u>Anodonta grandis</u>
Three-ridge	<u>Amblema plicata</u>
Pocketbook	<u>Lampsilis cardium</u>
Mapleleaf	<u>Quadrula quadrula</u>
Washboard	<u>Megaloniaias nervosa</u>
Yellow sandshell	<u>L. teres</u>
Pimpleback	<u>Q. pustulosa</u>
Pink heelsplitter	<u>Potamilus alatus</u>
White heelsplitter	<u>Lasmigona complanata complanata</u>
Fragile papershell	<u>Leptodea fragilis</u>
Deertoe	<u>Truncilla truncata</u>
Hickorynut	<u>Obovaria olivaria</u>
Rock-pocketbook	<u>Arcidens confragosus</u>
Threehorn wartyback	<u>Obliquaria reflexa</u>

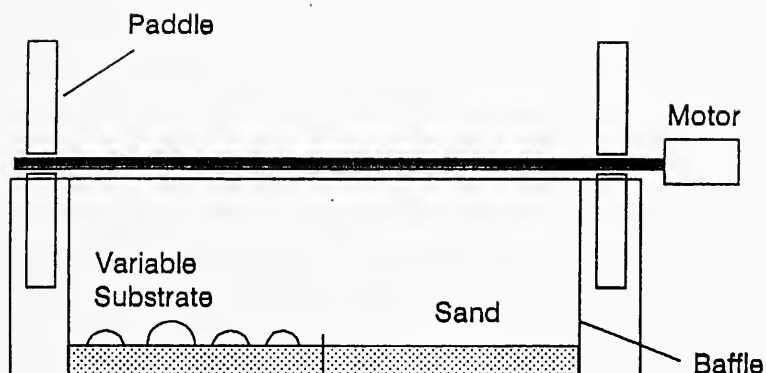


Figure 2. End-on view of substrate preference test tank. Variable substrate (rocks or mussels) is shown on the left side. Sand covers the bottom of the tank. Baffles account for the parallax of camera lens and keep fish in the camera's field of view. Water is circulated by motor driven paddles which move in the outer chamber created by the baffles.

again. This cycle continued until each fish was exposed to all substrates. Data collection began immediately after new substrates were placed in the tank.

We randomized the order and side of substrate presentation with large fish but with the small fish ran the control trial first. The variable substrates were then always placed opposite the preferred side, though the order of variable presentation was still randomized.

Data analysis. We used three-day substrate trials with bluegill, one-plus channel catfish, white crappie, sauger and the first set of fathead minnow trials. Analysis of the preference data indicated that some of the fish exhibited a preference on day one which diminished or changed to avoidance after three days perhaps in response to elimination of perizoon colonizing the surface of the substrate. For this reason, and to economize on time, data were collected for only one day with YOY walleye, channel catfish and largemouth bass and the second and third set of fathead minnow trials. We analyzed both one-day and three-day results of species which were used in the three-day trials.

Analysis of species substrate preference is based on the value of the relationship of two measures:

A = The percent of time the fish spent over the variable substrate.

B = The percent of time the fish spent over the corresponding side of the tank in the control trial.

Relating the measures in either of two ways, illustrated as 'a' or 'b' below, determines how much increase or decrease in time spent over the variable substrate is possible compared to the control trial.

$$\text{a) } \frac{(A - B)}{(1 - B)} \qquad \text{b) } \frac{(A - B)}{B}$$

If A is greater than B, relationship 'a' indicates how much the time spent over the variable increased beyond the time that was spent over that side of the tank in the control trial relative to the increase in time possible for that side of the tank. The outcome measure is positive and ranges from near zero to positive one. If A is less than B relationship 'b' indicates how much less time was spent over the variable compared to what was spent in the control trial relative to the decrease in time possible for that side of the tank. The outcome measure of this relationship is negative ranging from near zero to negative one.

We used a one-sample t test to compare the mean for each outcome measure to zero. If there was little or no difference between time spent over the variable versus the control the difference between the two percentages would be nearly zero. Conversely, if time spent over the variable was much more or less than time spent over the control the difference between the two percentages could be significantly different from zero. We used a treatment by time (3 or 4 X 2) repeated measures analysis of variance with a simple main effects test on treatment and time.

## SUBSTRATE PREFERENCE RESULTS

We use the term "prefer" to describe a relative increase in time spent over the variable compared to the control and the term "avoid" to refer to a decrease in that measure. Significant preference or avoidance indicates the measure differed significantly from zero ( $P < 0.05$ ).

In general responses of the fish to a substrate were stronger the first day than after three days. Though the majority of responses by the fish to the substrates were not significant, the overall frequency of preference or avoidance responses to a substrate showed some interesting trends.

Colonized mussels. Colonized mussels were preferred for at least part of the test period for all groups. The responses to this substrate were the strongest for all substrates tested, consequently this substrate elicited more significant responses than any other substrate (Figure 3).

Over the three-day trial period sauger exhibited a significant preference for colonized mussels during the day. Bluegill and white crappie in the small tanks also preferred colonized mussels though the responses were not significant (Figure 4).



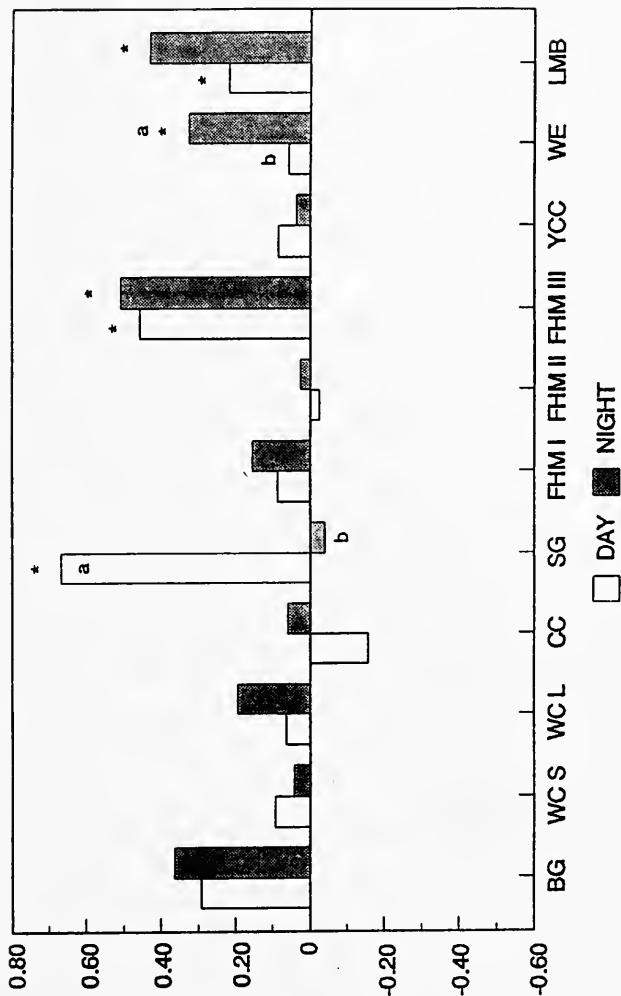


Figure 3. Response to colonized mussel substrate by all groups tested for the first day of the trial. Positive values indicate preference, negative values avoidance. \* Response to the substrate significantly different from zero ( $P < 0.05$ ). Columns with different letters within a species are significantly different. BG - bluegill; WCS - white crappie small tank; WCL - white crappie large tank; CC - juvenile channel catfish; SG - sauger; FHM I, II, III - fathead minnow trials one, two and three; YCC - young channel catfish; WE - young walleye; LMB - young largemouth bass.

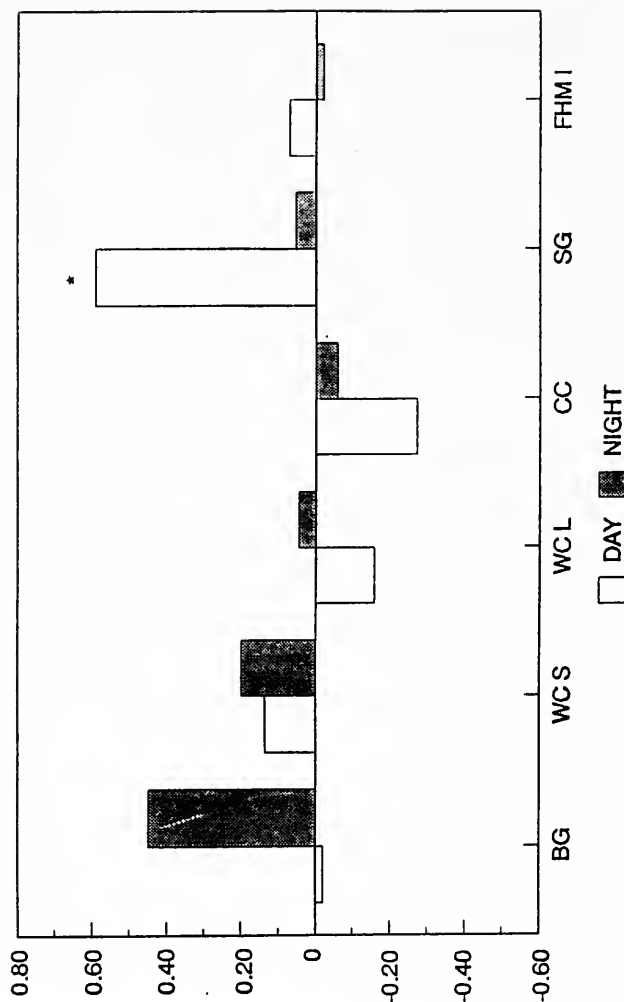


Figure 4. Response to colonized mussels after three days. Positive values indicate preference, negative values avoidance. \* Response to the substrate significantly different from zero ( $P < 0.05$ ). BG - bluegill; WCS - white crappie small tank; WCL - white crappie large tank; CC - juvenile channel catfish; SG - sauger; FHM I - fathead minnow trial one; YCC - young channel catfish; WE - young walleye; LMB - young largemouth bass.

Scrubbed mussels. Scrubbed mussels were not preferred as frequently as colonized mussels nor were the responses of the fish significant in as many cases (Figure 5). Over the three-day trial period there were no significant responses to scrubbed mussels from any of the fish tested (Figure 6). Though the bluegill differed in response to the substrate between the first and third day, in general the response of each species to the substrate after three days was similar to the response the first day of the trial. Again the response exhibited by the fish after three days was weaker than the response to the substrate the first day.

Clean rocks. With the exception of white crappie in the large tanks, the groups of fish most frequently exhibited an avoidance response to the clean rocks. The white crappie preferred the clean rocks both day and night, while sauger and young channel catfish preferred the rocks only at night. None of the preferences were significant, but juvenile channel catfish and the second group of fathead minnows exhibited significant avoidances of clean rocks (Figure 7).

The response of the fish to clean rocks varied over the three-day trial period. After three days all groups exhibited a preference for the clean rocks during day or night. Juvenile channel catfish continued to exhibit a significant avoidance of clean rocks during the day (Figure 8).

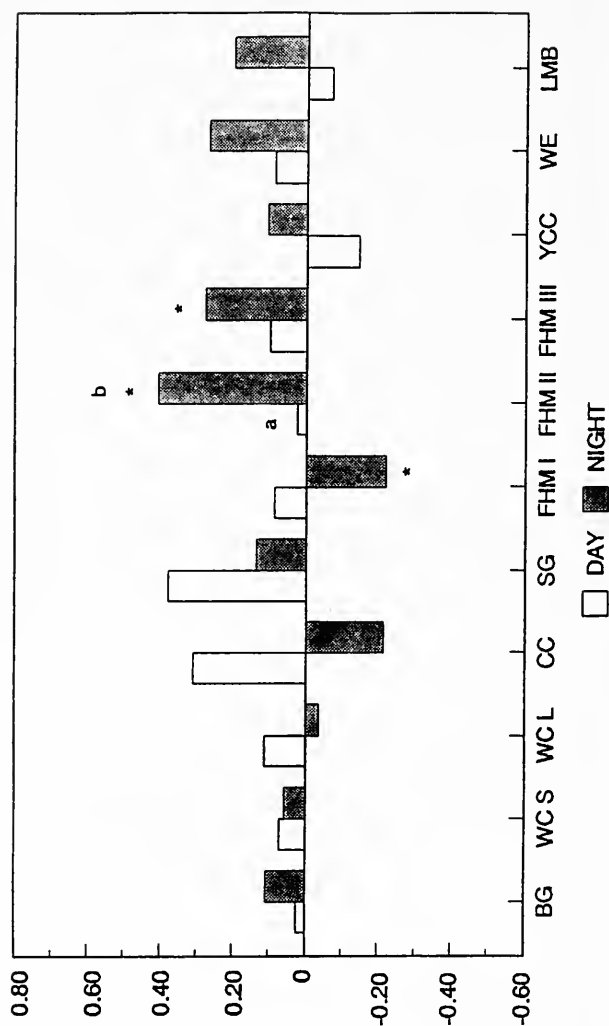


Figure 5. Response to scrubbed mussels by all species for day one. Positive values indicate preference, negative values avoidance. \* Response to the substrate significantly different from zero ( $P < 0.05$ ). Columns with different letters within a species are significantly different. BG - bluegill; WCS - white crappie small tank; WCL - white crappie large tank; CC - juvenile channel catfish; SG - sauger; FHM I, II, III - fathead minnow trials one, two, and three; YCC - young channel catfish; WE - young walleye; LMB - young largemouth bass.

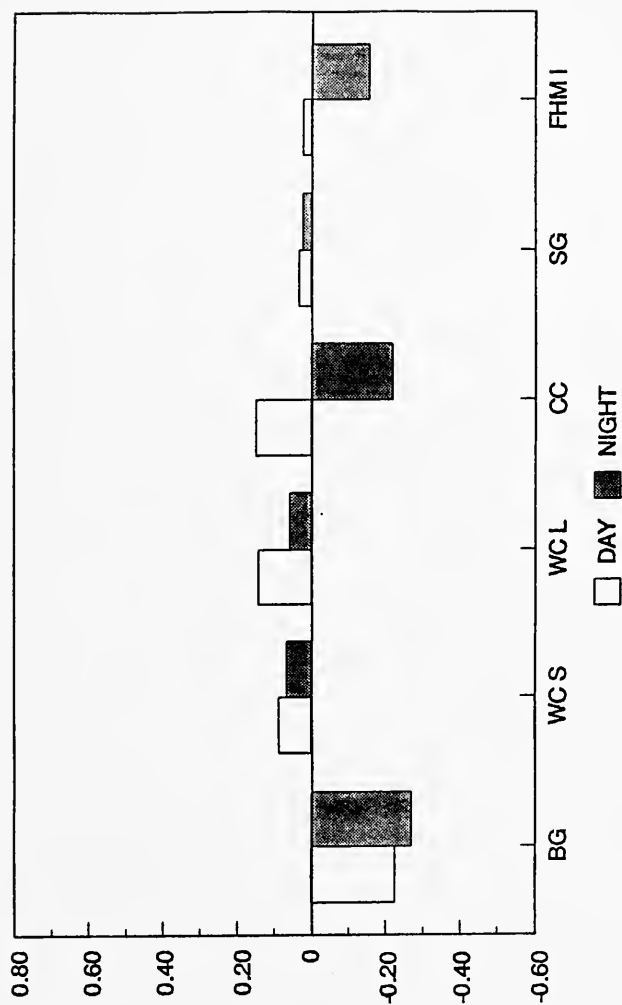


Figure 6. Response to scrubbed mussels after three days. There were no significant responses to the substrate. Positive values indicate preference, negative values avoidance. BG - bluegill; WCS - white crappie small tank; WCL - white crappie large tank; CC - juvenile channel catfish; SG - sauger; FHM I - fathead minnow trial one; YCC - young channel catfish; WE - young walleye; LMB - young largemouth bass.

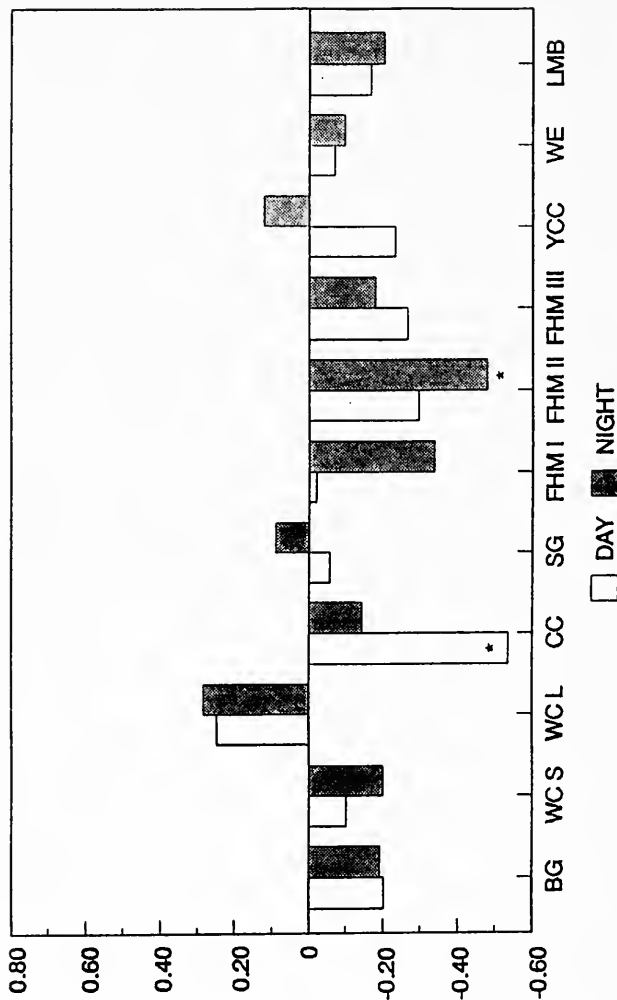


Figure 7. Response to clean rocks for day one. Positive values indicate preference, negative values avoidance. \* Response to the substrate significantly different from zero ( $P < 0.05$ ). BG - bluegill; WCS - white crappie small tank; WCL - white crappie large tank; CC - juvenile channel catfish; SG - sauger; FHM I, II, III - fathead minnow trials one, two, and three; YCC - young channel catfish; WE - young walleye; LMB - young largemouth bass.

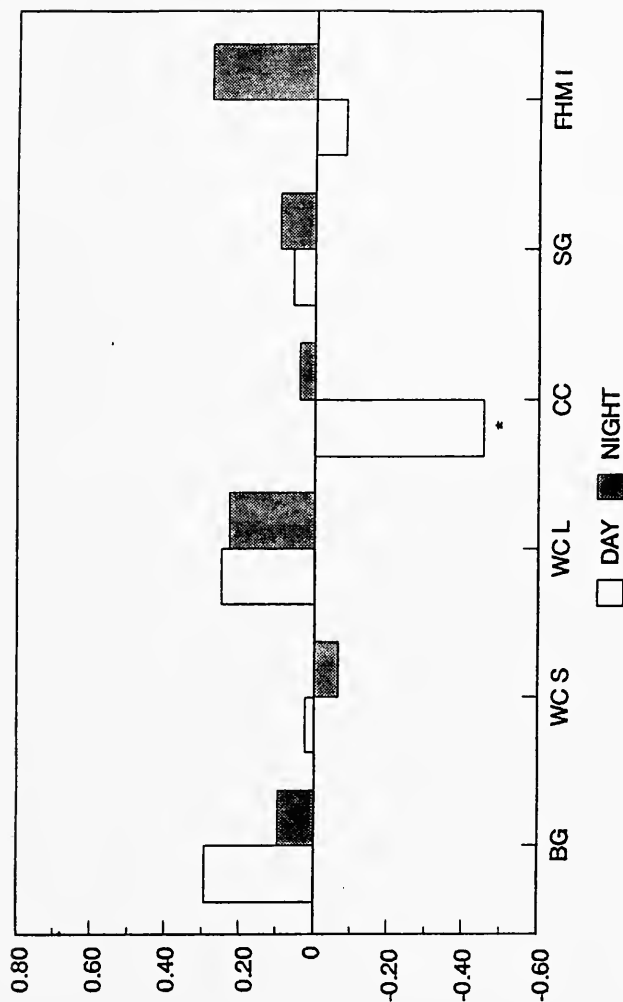


Figure 8. Response to clean rocks after three days. Positive values indicate preference, negative values avoidance. \* Response to the substrate significantly different from zero ( $P < 0.05$ ). BG - bluegill; WCS - white crappie small tank; WCL - white crappie large tank; CC - juvenile channel catfish; SG - sauger; FHM I - fathead minnow trial one; YCC - young channel catfish; WE - young walleye; LMB - young largemouth bass.

Colonized rocks. Colonized rocks were tested with five groups of fish, two groups of fathead minnows, and all the young-of-the-year fishes (walleye, channel catfish and largemouth bass). Figure 9 compares the responses to clean and colonized rocks exhibited by these five groups. The largemouth bass and two groups of fathead minnows exhibited the greatest differences in their responses to these substrates; these species preferred colonized rocks and avoided the clean rocks. The young channel catfish were mixed in their responses to these substrates and walleye avoided both substrates though the avoidance of clean rock was slightly stronger than the avoidance of colonized rock.

Dead shells. Only three species were tested over the dead shell substrate: bluegill, white crappie in the small tanks and juvenile channel catfish. Figure 10 compares the response of these groups to this substrate the first and third days of the trial. Bluegill and channel catfish preferred this substrate both day and night for the duration of the trial. Conversely, white crappie exhibited a significant avoidance of this substrate both day and night through the third day.



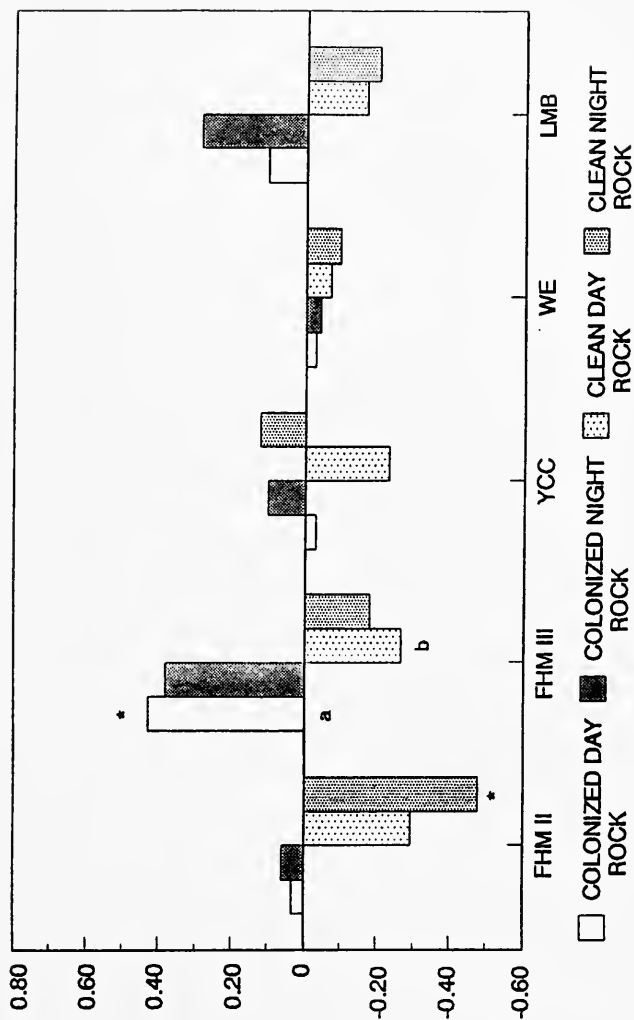


Figure 9. Response of fish to colonized versus clean rock. Positive values indicate preference, negative values avoidance. \* Response to the substrate significantly different from zero ( $P < 0.05$ ). Columns with different letters within a species are significantly different. FHM II, III - fathead minnow trials two and three; YCC - young channel catfish; WE - young walleye; LMB - young largemouth bass.

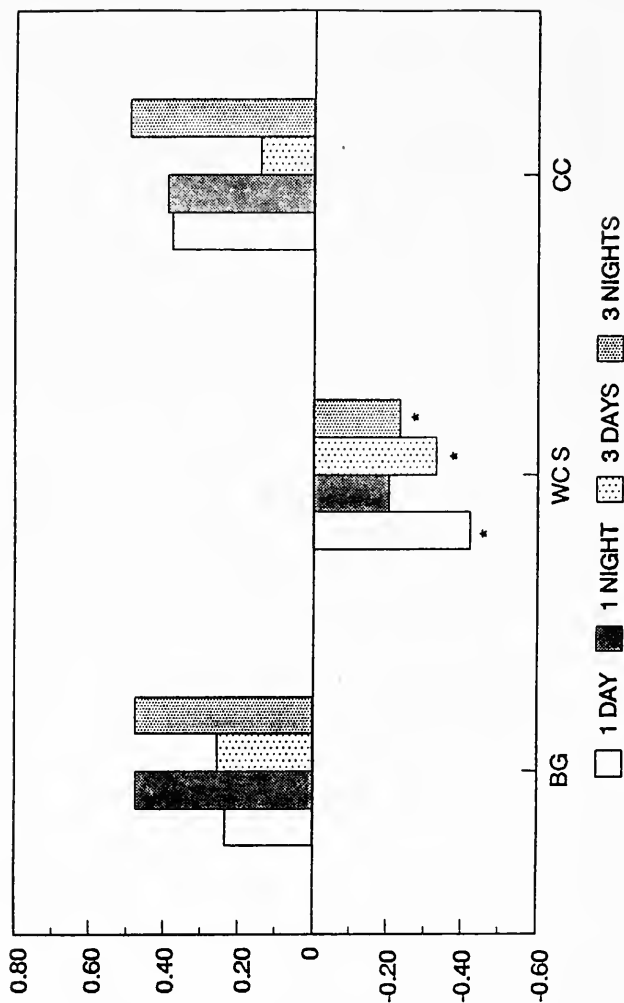


Figure 10. One-day and three-day response of fish to dead shells substrate. Positive values indicated preference, negative values avoidance. \* Response to the substrate significantly different from zero ( $P < 0.05$ ). BG - bluegill; WCS - white crappie small tank; CC - juvenile channel catfish.

#### SUBSTRATE PREFERENCE DISCUSSION

Overall the results from the preference trials indicate that the fish preferred mussels more frequently than clean rock. Table 5 presents the preference results from day one of all trials. Colonized and scrubbed mussels were preferred more frequently than clean rocks, and no species exhibited a significant negative response to these two substrates but clean rocks were significantly avoided once both day and night. Though colonized rock was tested in only five trials, this substrate was preferred more frequently than clean rock and was never avoided. Most of the preferences and avoidances were not significant though fish most frequently displayed a significant preference for colonized mussels followed by scrubbed mussels and colonized rock. Clean rocks were avoided most frequently and dead shells, which were tested in only three trials, produced mixed results (Table 6). Figure 11 illustrates the cumulative frequency of preference and avoidance during the first day of the substrate preference trials for each species.

In the five trials with colonized rock, this substrate was never avoided and was preferred in several cases. In these same five trials, clean rocks were avoided by four species and the fifth species avoided clean rocks during the day.

Table 5. Summary of (day,night) results from the substrate preference trials for all species. + preference for the substrate, - avoidance of the substrate, = increase or decrease from the control was less than five percent. ( ) under scrubbed mussels indicates response to scrubbed mussels where sand was the variable. nt - not tested.

Species	Colonized Mussels	Scrubbed Mussels	Clean Rock	Colonized Rock	Dead Shells
Bluegill	=,+	(-,-)	+,+	nt	+,+
Bluegill <sup>a</sup>	+,+	(=,+)	-*	nt	+,+
1+ C. Catfish	-,-	(+,-)	-*,=	nt	+,+
1+ C. Catfish <sup>a</sup>	-,+	(+,-)	-*, -	nt	+*, *
W. Crappie small	+,+	(+,+)	=,-	nt	-*, -
W. Crappie small <sup>a</sup>	+,=	(+,+)	-,-	nt	-*, -
W. Crappie large	-,-	+,+	+,+	nt	nt
W. Crappie large <sup>a</sup>	+*,+	+,=	+,+	nt	nt
Sauger	+*,+	=,=	+,+	nt	nt
Sauger <sup>a</sup>	+,=	+,+	-,+	nt	nt
Fathead Minnow I	+,=	=,-	-,+	nt	nt
Fathead Minnow I <sup>a</sup>	+,+	+,-*	=,-*	nt	nt
Fathead minnow II	=*,=	=,+,*	-,-	=*,+	nt
Fathead Minnow III	+,+,*	+,+	-,-	+,+,	nt
YOY C. Catfish	+,=	-,+	-,+	=,+	nt
YOY Walleye	+*,+,*	+,+	-,-	=,=	nt
YOY Largemouth Bass	+,+,+	-,+	-,-	+,=	nt

\*) Significant preference or avoidance.

a) First day of three-day trial.

YOY) Young-of-the-year fish.

Table 6. Summation of responses to the substrates for all species (from table 5). The first digit in each pair of numbers is the sum of the responses during the day for the indicated substrate. The second digit is the sum of the nocturnal responses. The paired digits in brackets represent the number of significant [day, night] responses. Equal means less than 5 percent difference between the response to the substrate and the control.

Day 1 of 3-day trials

Response	Colonized Mussels	Scrubbed Mussels	Clean Rock	Colonized Rock	Dead Shells
Prefer	5,4 [1,0]	5,3 [0,0]	1,2 [0,0]	not tested	2,2 [0,0]
Avoid	1,0 [0,0]	0,2 [0,0]	4,4 [1,0]		1,1 [1,0]
Equal	0,2	1,1	1,0		0,0

Day 3 of 3-day trials

Response	Colonized Mussels	Scrubbed Mussels	Clean Rock	Colonized Rock	Dead Shells
Prefer	3,3 [1,0]	3,2 [0,0]	3,4 [0,0]	not tested	2,2 [0,0]
Avoid	2,1 [0,0]	1,3 [0,0]	2,1 [1,0]		1,1 [1,1]
Equal	1,2	2,1	1,1		0,0

Day 1 of all trials

Response	Colonized Mussels	Scrubbed Mussels	Clean Rock	Colonized Rock	Dead Shells
Prefer	9,7 [3,3]	7,8 [0,2]	1,3 [0,0]	2,3 [1,0]	2,2 [0,0]
Avoid	1,0 [0,0]	2,2 [0,0]	9,8 [1,1]	0,0 [0,0]	1,1 [1,0]
Equal	1,4	2,1	1,0	3,2	0,0

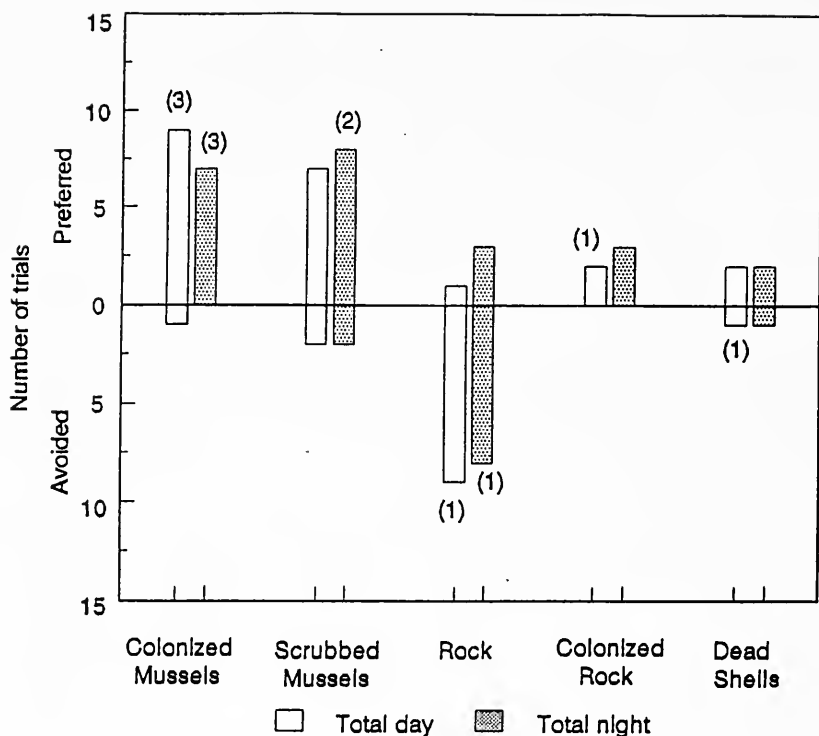


Figure 11. Frequency of substrate preference for all species in the substrate preference trials. The numbers in parentheses above and below the bars indicate the number of significant preference and avoidance responses respectively for each substrate ( $P < 0.05$ ). Responses less than 5% different from the control are not counted.

Colonized rock was never avoided by the five species tested with this substrate. Clean rocks were avoided by four of these five species both day and night and the fifth species avoided the clean rocks during the day. This suggests the fish were responding to forage or an odor associated with the colonized substrates. The clean rocks and dead shells were stored in a dry container prior to use in the substrate preference trials; these substrates were not colonized by bacteria or invertebrates before being placed in the test tanks. Since the rocks were not colonized by bacteria or invertebrates, the clean rocks may have had an unusual odor or lack of odor which caused the fish to avoid this substrate. Marzolf (1966) noted the amphipod Pontoporeia affinis showed significant selection of substrates whose surfaces had been "conditioned" by organic matter or bacteria.

## PREDATOR - PREY TRIALS

### METHODS

On two consecutive days, we placed five fathead minnows in each of three 48-inch-wide substrate preference tanks with a sauger. We performed all trials under daytime illumination with (overhead lamps on) sand on one side and rocks on the opposite side of the tank.

We visually observed the fish until all the fathead minnows were consumed or for one hour, whichever occurred first. We generally recorded whether each fish was on the left or right side once each minute. The locations of the fish were recorded more frequently if the fish changed sides or there was a feeding event, less frequently if the fish did not move or there was no interaction.

Analysis. We counted the number of minnows captured over each substrate,  $C_s$  for sand and  $C_r$  for rock and the time each species spent over the two substrates in the trials,  $T_s$  for sand and  $T_r$  for rock. For both species we multiplied the number of minutes for each time interval by the number of fish present on a given side of the tank for each time interval. This created a unit we call a fish-minute. There was only one sauger per tank so predator fish-minutes equal the total observation time. For fathead minnows we multiplied the number of fish remaining at each interval by the length of the interval and totaled the result.



The trials were of different lengths because the sauger consumed the fathead minnows more rapidly in some trials than in others. To give each trial equal weight in the analysis we calculated the mean trial length in fish-minutes for each species and divided this mean by the length of each trial. The mean duration of the trials for sauger was 35 fish-minutes; for fathead minnows the mean duration was 72 fish-minutes. Division of these values by the duration of each trial for each species produces a weighting factor. This factor is less than one for trials longer than the mean, greater than one for trials shorter than the mean. When the weighting factor is multiplied by the fish-minutes spent over each substrate ( $T_s$  or  $T_r$ ) in a given trial, the result is the relative time the fish spent over the substrates in trials of equal duration (Appendix E).

For each trial and species we also calculated the percent of the total fish-minutes spent over each substrate,  $\%T_s$  for sand or  $\%T_r$  for rock. Percent of captures over each substrate is represented by  $\%C_s$  for sand or  $\%C_r$  for rock. We analyzed both arcsin-transformed data and raw percent data; the transformed data produced similar results, therefore only the raw percent data are presented.

We used a one-sample t test to examine whether the mean of the difference between the number of captures over the two substrates was significantly different from zero; the same test was used for fish-minutes.

$$H_0: \frac{\sum(C_s - C_r)}{n} = 0 \quad \text{and} \quad H_0: \frac{\sum(T_s - T_r)}{n} = 0$$

We also used a one-sample t test to examine whether the mean percent of captures and mean percent time over each substrate were significantly different from 50 percent.

$$H_0: \% \bar{C}_s \text{ or } \% \bar{C}_r = 50 \% \text{ and } H_0: \% \bar{T}_s \text{ or } \% \bar{T}_r = 50 \%$$

We calculated captures per fish-minute (C/T) for each species by substrate. This measure indicates the substrate-dependent minnow capture rate for the sauger and a substrate-dependent escape factor for the minnows. A high value indicates more minnows were captured per fish-minute. The values for each substrate were compared with ANOVA by species.

PREDATOR-PREY  
RESULTS AND DISCUSSION

Sauger spent slightly more time over sand than over rock substrates. Fathead minnows spent twice as much time over rock than over sand. For both species the mean difference in fish-minutes spent over the two substrates was not significantly different from zero and the percent time spent over either substrate was not significantly different from 50 percent. Arcsin-transformed data produced similar results.

Eighty-three percent of the minnow captures occurred over the sand substrate, significantly more than over rock. However, the mean difference between the number of captures over each substrate was not significantly different from zero and the percent of captures over each substrate did not differ significantly from 50 percent. The rate of minnow capture by sauger (captures/sauger-minute) and the fathead minnow mortality rate (captures/minnow-minute) did not differ significantly between the two substrates (Table 7).

The minnows spent significantly more time over rock than over sand yet 83 percent of the total captures occurred over sand. The rate of minnow capture by sauger was approximately three times greater over sand than over rock, 0.26 and 0.09 respectively. The fathead minnows were twelve times more vulnerable over sand than over rock. Minnow mortality per minnow-minute was 0.25 over sand and 0.02 over rock, though the difference was not significant ( $P > 0.05$ ).

Table 7. Mean time (T) spent over rocks and sand by sauger and fathead minnows, percent of minnow capture and capture per fish-minute by substrate in the predator-prey study.

Sauger

Substrate	Mean Minnows Captured	Percent Minnows Captured	Fish-minutes (T)	Percent T	Catch per Fish-minute
Sand	3.83 *	83.3 *	20.8	59.5	0.26
Rock	0.83	16.7	14.2	40.5	0.09

Fathead Minnow

Substrate	Mean Minnows Captured	Percent Minnows Captured	Fish-minutes (T)	Percent T	Mortality per Fish-minute
Sand	3.83 *	83.3 *	23.7 *	32.9 *	0.25
Rock	0.83	16.7	48.3	67.1	0.02

\* Significant difference between substrates ( $P < 0.05$ ).

We compared the percent of time spent by each species over each substrate in the rock - sand preference trials with the percent time spent over the 'same' substrates in the predator-prey trials using ANOVA. We used both arcsin-transformed and raw percent data for the analyses: since arcsin-transformed data produced the same result, untransformed data are presented.

In the third day of the preference study, where behavior was compared to a sand-sand control, sauger exhibited a slight preference for rock in the rock-sand trial. Comparison of the percent time spent on either side of the tank within the rock-sand trial, as in the predator-prey study, indicates the fish spent a greater percent of the time over rock though the difference was not significant. There was no significant difference in time spent over a given substrate between the predator-prey and preference trials.

The preference measure used in the substrate preference study indicated the fathead minnows frequently avoided clean rock. The ANOVA test of percent of time spent on each side of the tank in the sand - rock trial also indicated the fish spent more time over sand than rock, significantly more in trial three. In the predator-prey trials however, the minnows spent significantly more time over rock than over sand. Percent time spent by fathead minnows over a given substrate was significantly different between the predator-prey trials and trial three of the substrate preference study (Figure 12).

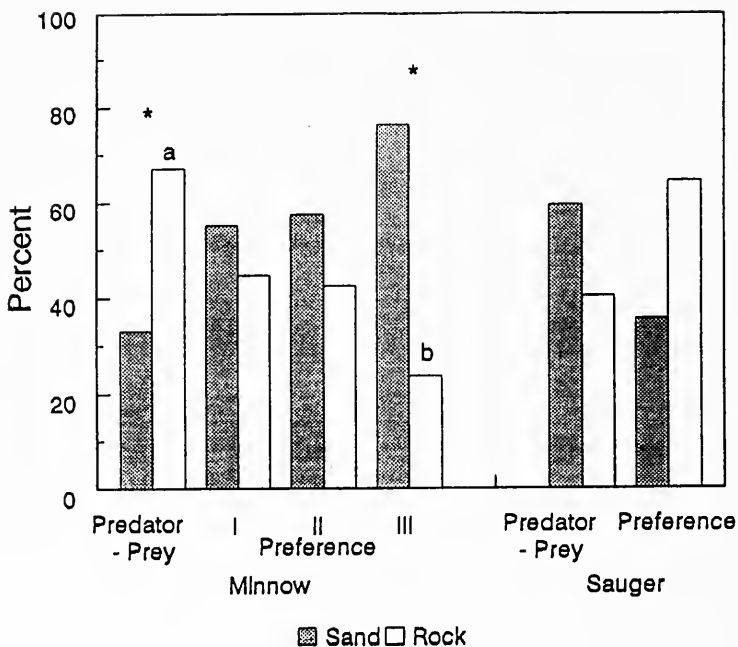


Figure 12. Comparison of percent time spent over rock and sand by sauger and fathead minnow in the predator-prey study and the substrate preference trials. \* Significant difference between the response to sand and rock within a trial. Different letters indicate a significant difference in response to a substrate between trials ( $P < 0.05$ ). Unmarked columns within a substrate are not significantly different. (Differences in minnow response to sand were also significant, but opposite the response to rock).

Examination of the inverse of the capture rate provides some interesting information. The sauger spent an average of 3.8 minutes per minnow capture over sand and 11 minutes over rock. The fathead minnows spent 4 fish-minutes over sand for each one captured, but spent an average of 50 fish-minutes over rock before a minnow was captured. These results indicate that the irregular bottom profile formed by rocks in the experimental tanks provided the fathead minnows refuge from predation by the sauger. This finding suggests that the irregular bottom profile of a mussel bed could provide forage fish with some refuge from predation.

These results, though limited in scope, agree with those of other predator-prey studies. Prey shift their distribution in response to a predator (Fraser and Cerri 1982, Stein 1979, Stein and Magnuson 1976) and predators are more efficient in less complex habitats (Stein 1977, Crowder and Cooper 1982). It seems reasonable that these predators could forage on the edge of a cobble habitat and thus be provided with both a better chance for prey capture over the open substrate as well as higher prey population densities over the cobble substrate.

## FEEDING TRIALS

### MATERIALS AND METHODS

During the substrate preference trials we observed fathead minnows consume mussel feces. To investigate whether fathead minnows may derive nutritional benefit from mussel feces or pseudofeces, we held fathead minnows in tanks alone or with mussels. The only food available to the fish during the eight, two-week trials was silt, phytoplankton and small invertebrates which entered the tank in water pumped from a nearby creek.

Water Delivery. The feeding trials took place in 30 five-gallon aquaria contained in a 14 x 3.25 x 2.4-ft trough partially filled with water to form an isothermate water bath. Water was pumped from nearby Quiver Creek with a 1/4-hp submersible pump replaced later by a 1/2-hp centrifugal pump. Creek water entered a head tank in the laboratory for aeration and mixing. Pump operation was regulated by a float switch in the head tank which maintained the water level within a set range therefore the head pressure was relatively stable even though the two pumps differed in rate of water delivery. An airstone and turbulence caused by water entering the tank kept sediment and planktonic matter mixed for delivery to the tanks. The water was delivered by gravity flow through a control valve for distribution to the aquaria (Figure 13). Each aquarium had a screened siphon, a glass lid to help prevent escape of test fish and an airstone (Figure 14). Water added to the aquarium exited through the siphons into the water



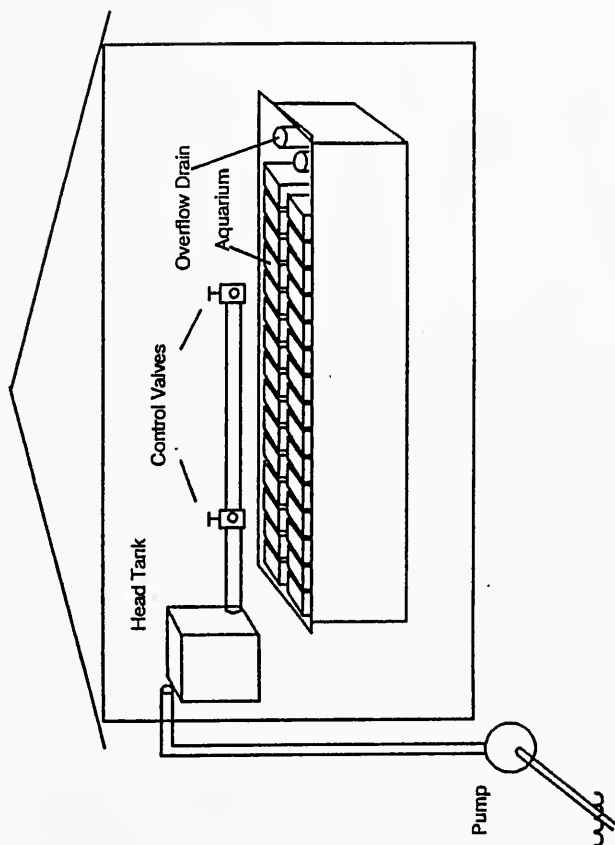


Figure 13. System design for the mussel feces feeding trials with fathead minnows. The trough containing the aquaria was housed indoors. Water was pumped from a nearby creek and entered a head tank for mixing and aeration. Water flowed by gravity to control valves and was delivered to the aquaria with a hand-held hose in low flow trials or with a manifold in high flow trials.

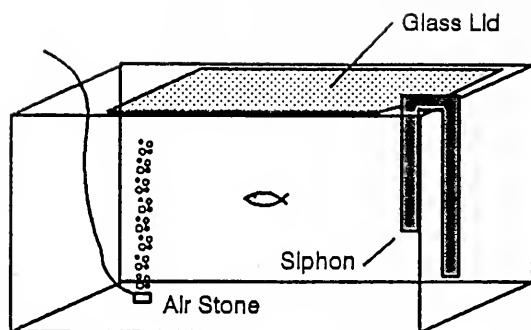


Figure 14. Detail of one of the thirty, five-gallon tanks used for the feeding trials.

bath in the surrounding trough.

The method and volume of water delivery to the tanks varied with the trials. In trials one, two and three, two gallons of water was delivered with a 3/4-inch diameter hose to each tank two times daily. Prior to adding water to the aquaria we ran the pump continuously for 15 minutes to ensure that water in the head tank was fresh and well mixed.

For the first five days of trial four, water was delivered to each tank as in the previous trials. During the remaining nine days of trial four and the full duration of trials five through eight, the water was delivered through a manifold to all tanks in a constant stream at a mean rate of 339 gallons per tank per day.

It was difficult to establish an equal flow to each tank with the manifold system and water flow varied significantly between tanks with highest and lowest flow rates. However, there was no significant difference in mean flow between treatments within a trial ( $P > 0.05$ ).

Water Treatment. In all trials except number four, the water was strained through a 0.01 inch-mesh net to remove most vegetation, zooplankton and insect larvae from the incoming water. In trial four the water was not strained. In trials one through three water was strained just prior to entry to the tanks at the end of the hose. In trials five through eight, when the manifold was used, water was strained prior to entry to the head tank.

Water Quality. We measured temperature, dissolved oxygen, pH, turbidity and total ammonia nitrogen daily in all tanks in trials three through eight. We used a Hach model FF-1A water quality test kit to measure ppm total ammonia nitrogen ( $\pm 0.1$  ppm). We measured turbidity with a Hach DRL model 2506-05 water quality kit; results are reported in Formazin turbidity units (FTU) ( $\pm 2.5$  FTU). Milligrams/liter total ammonia nitrogen was measured with a Hach model FF-1A water quality test kit ( $\pm 0.1$  ppm). This kit was also used to measure pH in trials one through three ( $\pm 0.5$ ). In trials four through eight, pH was measured with a Hanna pHep pocket pH meter ( $\pm 0.2$ ). The turbidimeter was obtained during the latter part of trial two, therefore turbidity was recorded for only a few days of this trial. Trial one was considered preliminary and no water quality data were collected.

Treatments. We used four basic treatments for the feeding trials: blank, mussel, fish, and mussel-fish. Variations on the mussel and mussel-fish treatments used thick- or thin-shell mussels. Trials variously used one or two mussels per tank and one or two fish per tank. The blank treatment provided a measurement of background sediment and protein values for each trial. Similarly the fish and mussel treatments provided a means of comparing sediment weights and protein values with the mussel-fish treatment.

Species. We used mapleleaf, three-ridge and floater mussels during the trials. Mapleleaf and three-ridge mussels are thick-shell

species, floaters are thin-shell mussels. Mapleleaf mussels were used in trial one only; three-ridge mussels were used in trials two and three, floaters were used in trials four through six and both three-ridge and floaters were used in trials seven and eight. We assumed that for a given wet weight, a thick-shell mussel would have less percent body tissue than a thin-shell mussel because more weight would be present as shell. Fathead minnow was the test fish in all trials. It was chosen for its availability and omnivorous food habits.

Replicates. For each of trials one through six there were five empty tanks, five containing mussels, ten containing fish, and ten containing mussels and fish together. In trials seven and eight we used ten tanks containing fish, and twenty containing mussels and fish. Of the twenty mussel-fish treatment tanks half were fish with thick-shell mussels, the other ten contained fish with thin-shell mussels. Even though the tanks had lids, fish were occasionally able to escape. Tanks from which fish escaped or into which fish jumped were omitted from analysis as were tanks where fish or mussel mortalities occurred; therefore sample sizes ranged from four to six in the trials.

Treatments were randomly assigned to tanks in the system. Mussels were scrubbed clean then weighed to  $2.2 \times 10^{-4}$  lb in water prior to being placed in the tanks. Fish were weighed to  $2.2 \times 10^{-5}$  lb in water before being placed in the test tank. In trials one through four we randomly assigned fish and mussels to tanks within a treatment. In trials five through eight fish or mussels were assigned to tanks

within a treatment according to a predetermined mussel:fish weight ratio to match or alter the ratio used in previous trials. Table 8 illustrates treatments and conditions used in each trial.

End of Trial. At the end of a feeding trial the water was turned off, fish were netted out and weighed as described above. We dried the fish overnight in an oven at 167-185 °F and measured dry weight with an analytical balance.

Since mussels were used in several non-consecutive trials, two conversion factors, one for thick-(three-ridge and mapleleaf) and one for thin-shell (floater) mussels were developed for calculation of dry tissue weight from wet, whole body weight. A sample of ten three-ridge and ten floater mussels was used to obtain the ratios of wet to dry weight. The mussels had been held indoors for 30 days, did not contain glochidia and appeared healthy. Each mussel was weighed wet, whole, then shucked and both wet body tissue and wet shell weight were recorded. Both shell and body tissue were dried overnight at 167-185 °F. Dry weights of shell and tissue were measured. Shells were weighed to  $2.2 \times 10^{-4}$  lb with an electronic balance; tissue weights were measured to  $2.2 \times 10^{-6}$  lb with an analytical balance.

Appendix F gives the wet and dry weights of the mussels used for calculation of percent dry tissue weight from whole body wet weight. Dry weight is estimated to be 2.02 percent and 1.54 percent of wet weight for three-ridge and floater mussels respectively but these means are not significantly different ( $P > 0.05$ ). The conversion

Table 8. Conditions and treatments for feeding trials.

Trial	°F	Mean Flow	Treatments <sup>a</sup>	Mussels/ Tank	Fish/ Tank	Mean lb Mussel/ lb fish <sup>b</sup>
1	64.4	2	B,C,F,K	1	1	0.035
2	74.3	2	B,C,F,K	2	1	0.093
3	76.1	2	B,C,F,K	2	1	0.088
4	74.1	219	B,M,F,N*	2	1	0.028
5	72.3	339	B,M,F,N	2	1	0.031
6	63.1	339	B,M,F,N	2	2	0.013
7	56.7	339	F,K,N	1	1	0.071, 0.016 <sup>c</sup>
8	51.4	339	F,K,N	1	1	0.064, 0.015 <sup>c</sup>

a) B - blank, C - thick-shell mussel, F - Fish, K - thick-shell mussel-fish, M - thin-shell mussel, N - Thin-shell mussel-fish.

b) Mussel:fish dry weight ratio.

c) Thick- and thin-shell mussel/fish weight ratios respectively.

Flow represents mean gallons/day water exchange per tank. Water was strained through a fine mesh net except as indicated by (\*). Trial 1 used mapleleaf mussels, the other trials with the thick-shell mussel treatment used three-ridge mussels.

factor for three-ridge mussels was applied to mapleleaf mussels.

We siphoned the silt and water from each tank at the end of the trial and filtered it through an  $3.1 \times 10^{-3}$  inch-mesh plankton net. We assumed fecal protein, in the form of feces deposited by fish and mussels, would be associated with the sediments and would wash into the receptacle at the bottom of the net. The filtrate was either frozen and dried later or immediately dried overnight at 167-185 °F. We measured dry sediment weights with an analytical balance ( $\pm 2.2 \times 10^{-7}$  lb) then stored the samples in a dessicator for the sediment protein analysis.

Sediment Protein. We used the micro-protein analysis procedure described by Smith et al. (1985) to determine if differences existed in the protein content of the sediment collected from different treatments. The assay, which measured protein rather than total nitrogen, was obtained from Pierce Chemical company.

The proteins must be solubilized for the assay. We used a one percent solution of sodium lauryl sulfate (SDS), a detergent, to solubilize the proteins associated with the sediment. In preliminary extraction trials we mixed 0.013 gal of SDS with 0.001-0.002 lb of sediment and placed the mixture in an oven at 140 °F overnight. Subsequently, we found 85 percent of the overnight extraction level could be achieved using the same solvent-to-sample ratio for four hours at 140 °F. Since our interest was in relative concentrations of sediment protein between treatments rather than absolute concentrations, we considered this level of extraction to be



satisfactory. Protein values are expressed as a concentration of protein per sediment (ppt).

Statistical analysis. We used the SAS PC general linear model (1985) analysis of variance procedure to determine if significant differences existed in fish weight change, percent fish weight change calculated as  $(\text{final weight} - \text{initial weight})/(\text{initial weight})$ , sediment protein concentration and water quality parameters between treatments. We used the SAS regression analysis to identify significant factors in the variance of percent fish weight change. As in the previous studies we analyzed both arcsin-transformed percent data and raw percent data. The results were similar, therefore the untransformed data are presented. The level of significance for all tests is  $P < 0.05$  unless otherwise noted.

## RESULTS OF FEEDING TRIALS

Fish weight change. Percent change in fish weight differed between treatments but was significantly different only in trials three and five (Figure 15). In trial three flow was two gallons per day. In this trial fish held with mussels lost less weight than fish held alone. In trial five, flow was 339 gallons per day; in this trial fish held with mussels lost weight while those held alone gained weight. Fish held with mussels in the other low flow trials (trials one and two) lost less weight than fish held alone but the differences were not statistically significant. In trials four and six, where flow was higher, fish held with mussels lost more or did not gain as much weight as fish held alone, though again the difference was not significant. In trials seven and eight where both thick and thin-shell mussel-fish treatments were used, the results were variable.

In trials with thick-shell mussels, fish held with mussels lost less weight than fish held alone except in trial eight. Trials two and three were performed under similar experimental conditions and fish in the same treatments in these two trials exhibited similar weight changes. Mean fish weight change was significantly different between treatments in trial three ( $P < 0.05$ ) but was not significantly different in trial two ( $P = 0.2062$ ). Pooling the weight change results from these two trials shows fish held with mussels lost significantly less weight than fish held alone ( $P = 0.0187$ ).

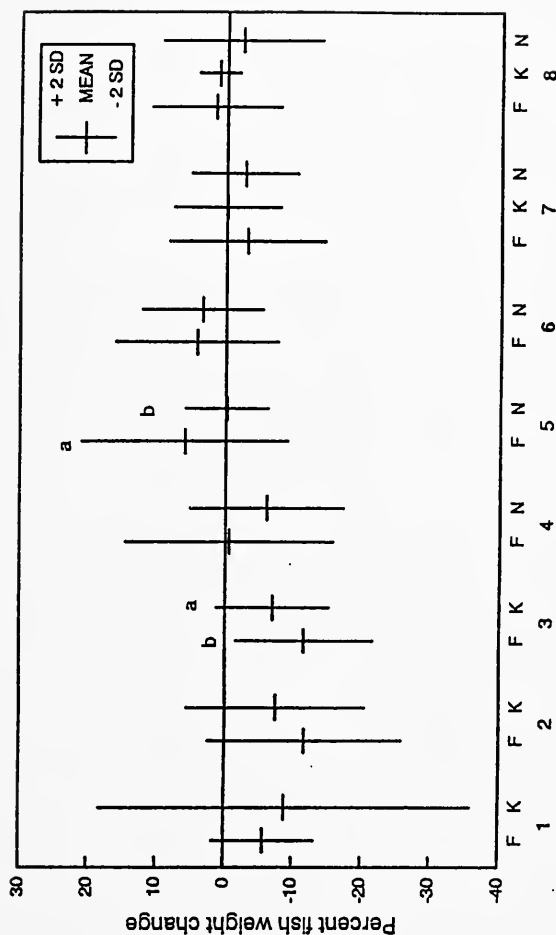


Figure 15. Percent weight change of fish in the feeding trials. Treatments with different letters within a trial are significantly different ( $P < 0.05$ ). Unmarked treatments are not significantly different. F - fish alone; K - fish held with thick-shell mussels; N - fish held with thin-shell mussels. Numbers refer to trials.

Sediment protein. In general, the sediment protein concentrations measured for the mussel-fish treatment in a trial were equal to or greater than the sediment protein concentrations from the fish treatment.

Sediment protein values tended to increase with higher water turnover (flow) in the tanks. Sediment protein varied significantly between treatments in trials one, three and five (Figure 16).

The sediment protein concentrations in the low flow trials were higher in the treatments with mussels than in tanks which held no mussels. In these trials the fish weight change seems to reflect the increased protein in the sediment, though the differences were not significant. In the high flow trials sediment protein concentrations tended to be somewhat higher in tanks with mussels, though this was not always the case as can be seen in trials four and eight, and fish weight change did not appear to reflect the increased protein levels available in the sediment.

In trial one, sediment protein concentration in the mussel treatment was higher than in all other treatments. In trial three the sediment protein concentration from the mussel treatment was significantly higher than treatments without mussels but not significantly greater than that measured for the mussel-fish treatment. The sediment protein concentration measured for the mussel-fish treatment in trial five was greater than the other three treatments but was significantly greater than the fish treatment.

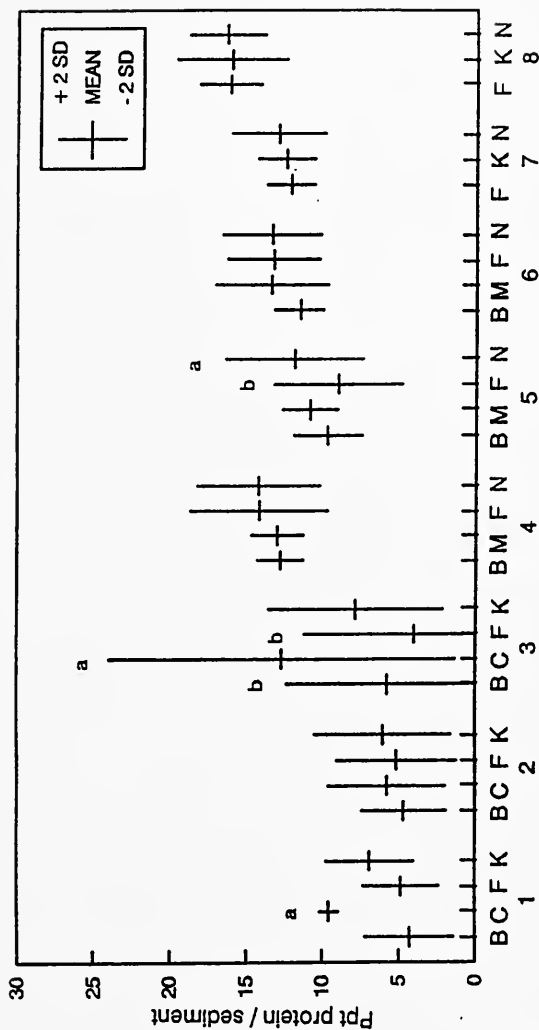


Figure 16. Mean sediment protein concentration (ppt protein/ sediment) for each treatment in the feeding trials. Different letters within a trial indicate significant differences ( $p < 0.05$ ). Unmarked treatments are not significantly different. B - blank; C - thick-shell mussel; F - fish held alone; K - fish held with thick-shell mussels; M - thin-shell mussel; N - fish held with thin-shell mussels. Numbers refer to trials.

Figure 17 illustrates that over all trials and treatments fish weight change has a weak positive but significant relationship with sediment protein concentration ( $r^2 = 0.188$ ). Note that Figure 17 illustrates a separation between fish weight change in the low and high flow trials. This suggests that fish weight change was responding to changes in flow. When split by treatment, this same relationship holds for fish weight change in the fish only treatment ( $r^2 = 0.284$ ) (Figure 18). The difference is significant ( $P < 0.05$ ) in the thick-shell mussel-fish treatment ( $r^2 = 0.362$ ) (Figure 19). The hypothesis that fish weight change responded to changes in flow is further supported by the results of the thin-shell mussel-fish treatments which were performed only at the high flow rate. Fish weight change from these treatments exhibits no significant relationship with sediment protein concentration and fish weight change ( $r^2 = 0.027$ ) (Figure 20). Recall from figure 15 that sediment protein concentrations increased with flow. Sediment protein concentration exhibits a significant positive relationship with flow ( $r^2 = 0.5390$ ) (Figure 21).

We calculated 'net' sediment protein to more clearly define the relationship between the protein contained in mussel feces, pseudofeces or fish feces and fish weight change. Net protein is calculated as:

$$P_{nij} = P_{gij} - P_{bj}$$

Where,

$P_{nij}$  = Net Protein for an individual tank 'i' in trial 'j'.

$P_{gij}$  = Gross Protein for an individual tank 'i' in trial 'j'.

$P_{bj}$  = Mean mg protein/g sediment for the blank treatment in trial 'j', to account for background protein.

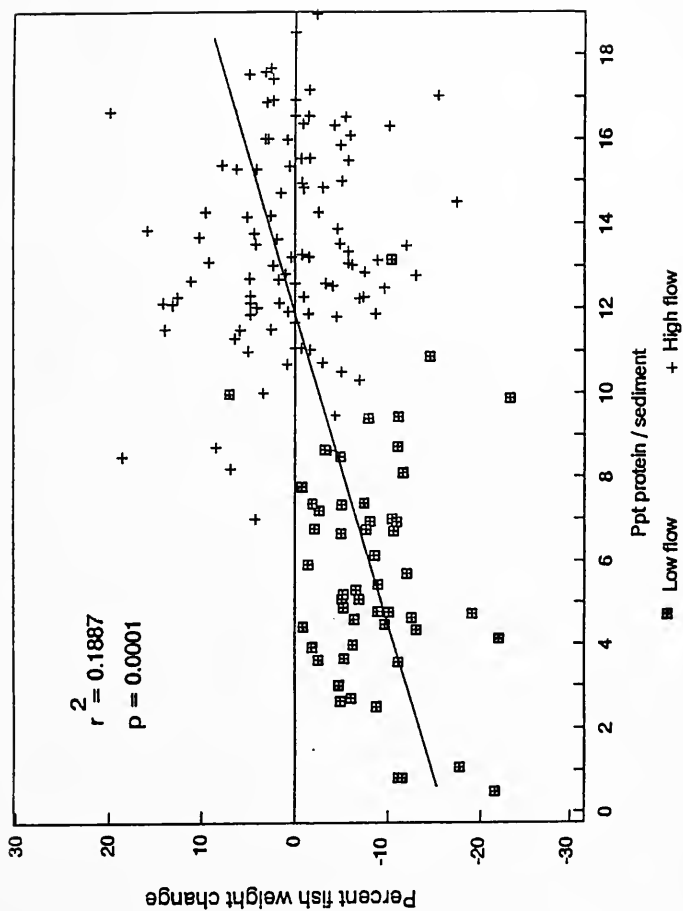


Figure 17. Regression of percent fish weight change with sediment protein concentration (ppt protein/sediment).

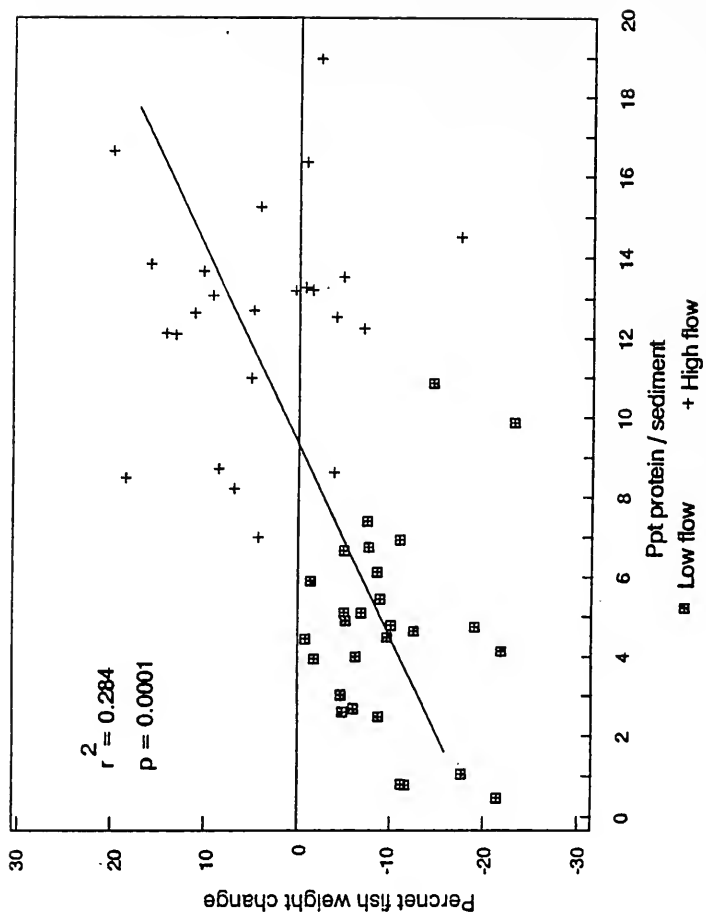


Figure 18. Regression of percent weight change of fish held alone with sediment protein concentration (ppt protein/sediment).



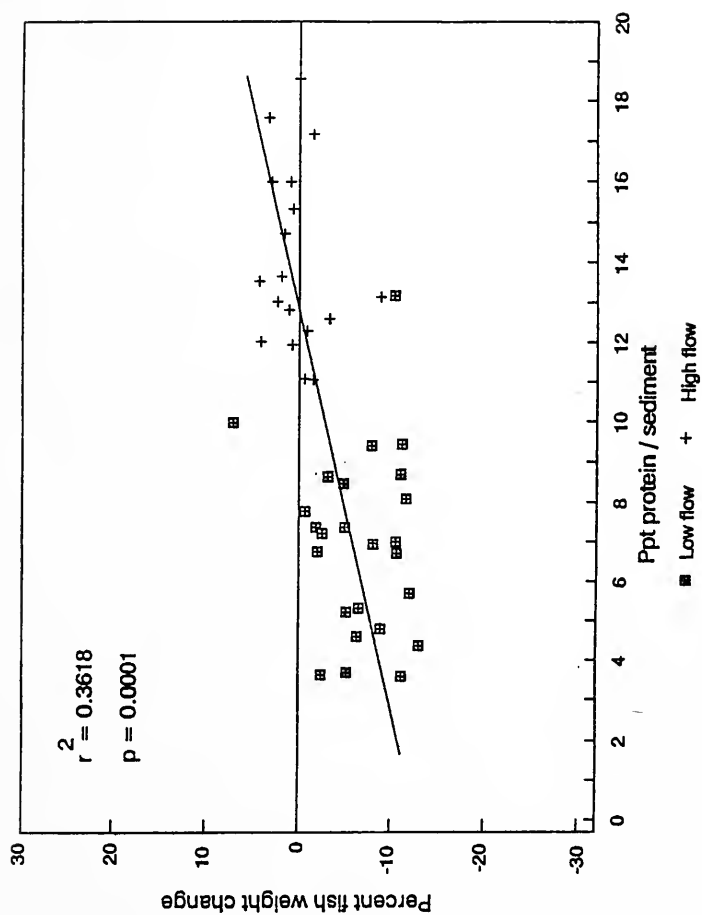


Figure 19. Regression of percent weight change of fish held with thick-shell mussels and sediment protein concentration (ppt protein/sediment).

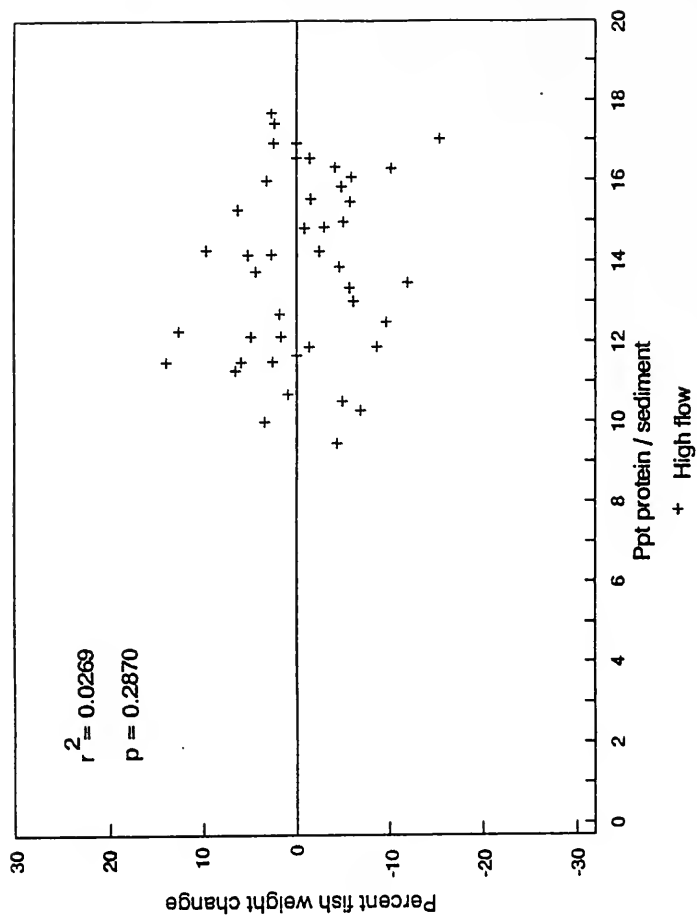


Figure 20. Regression of percent weight change of fish held with thin-shell mussels with sediment protein concentration (ppt protein/ sediment).

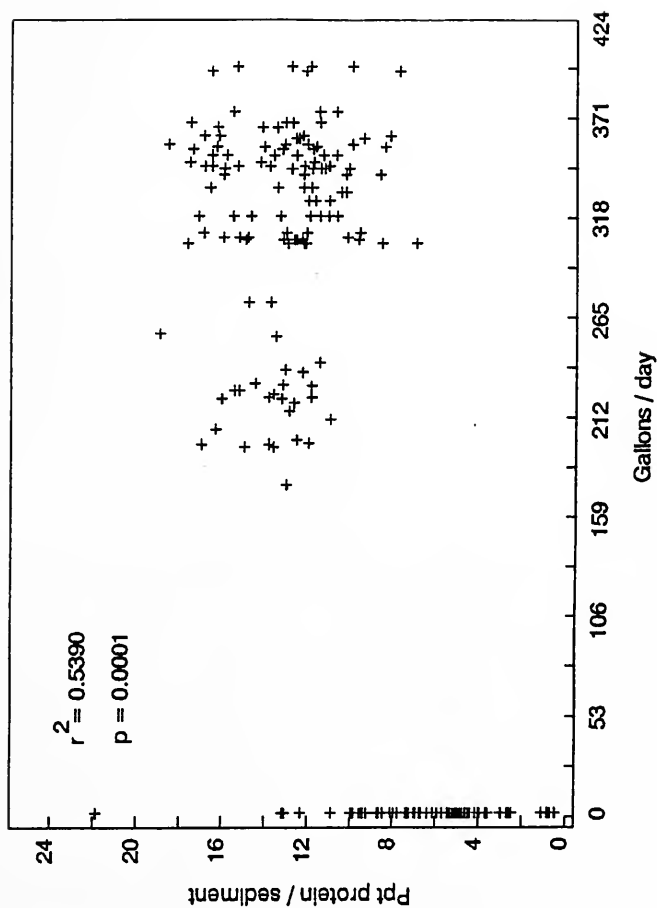


Figure 21. Regression of sediment protein concentration (ppt protein/sediment) with mean daily water exchange (gallons/day).

In trials seven and eight where there was no blank treatment we used the fish treatment as the background measurement. Calculation of these values provided a means of separating the effects of flow and sediment protein concentrations on percent fish weight change.

When the results of all trials were pooled by treatment, the mean net sediment protein concentration of the thick-shell mussel treatment was significantly greater than all other treatments (Figure 22) and there were no significant differences between the other treatments. The mussel treatment in trial one was significantly greater than both the fish and mussel-fish treatments but in trial three was not significantly greater than the mussel-fish treatment. In trial five the net sediment protein in the thin-shell mussel-fish treatment was significantly greater than the fish treatment.

The relationship between net sediment protein concentration and flow was not significant ( $r^2 = 0.008$ ) (Figure 23) nor was the relationship between net sediment protein and percent fish weight change ( $r^2 = 0.010$ ) (Figure 24). Water exchange rate (flow) accounted for thirty-two percent of the variance in fish weight change over all trials (Figure 25).

Comparison of the net sediment protein concentrations per gram of tissue for the three treatments (mussel, fish and mussel-fish) could show for example, whether thick-shell mussels produce higher sediment protein concentrations per gram dry tissue weight than thin-shell mussels. Figure 26 illustrates the results of dividing net sediment protein concentrations by total dry tissue weights for each treatment.

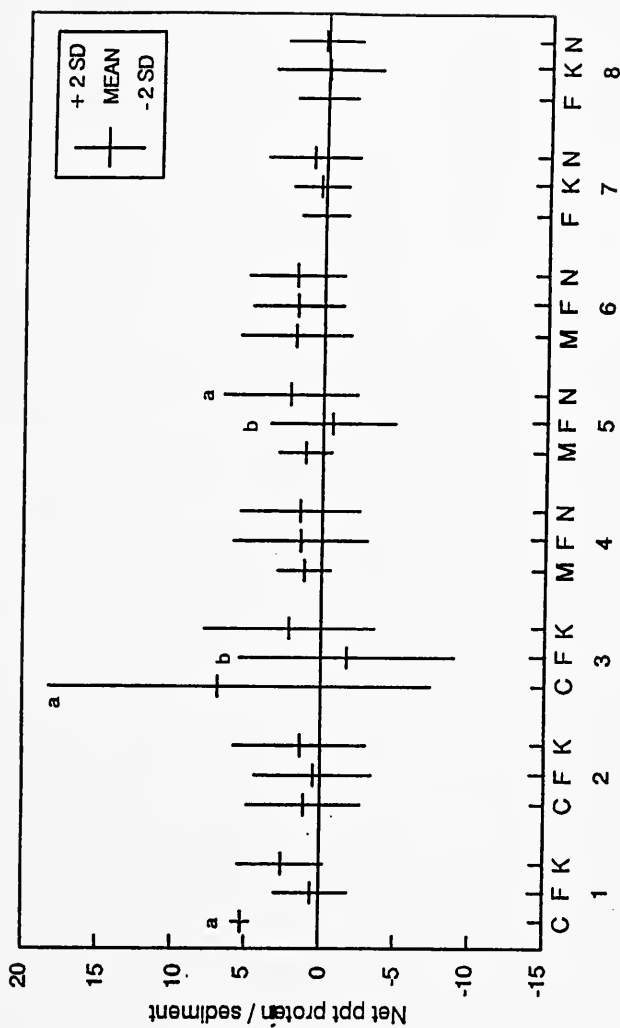


Figure 22. Net sediment protein concentration (ppt protein/ sediment) for each treatment in the feeding trials. Different letters indicate significant differences between treatments within a trial ( $P < 0.05$ ). Unmarked columns are not significantly different. C - thick-shell mussel; F - fish held alone; K - fish held with thick-shell mussels; M - thin-shell mussel; N - fish held with thin-shell mussels. Numbers refer to trials.

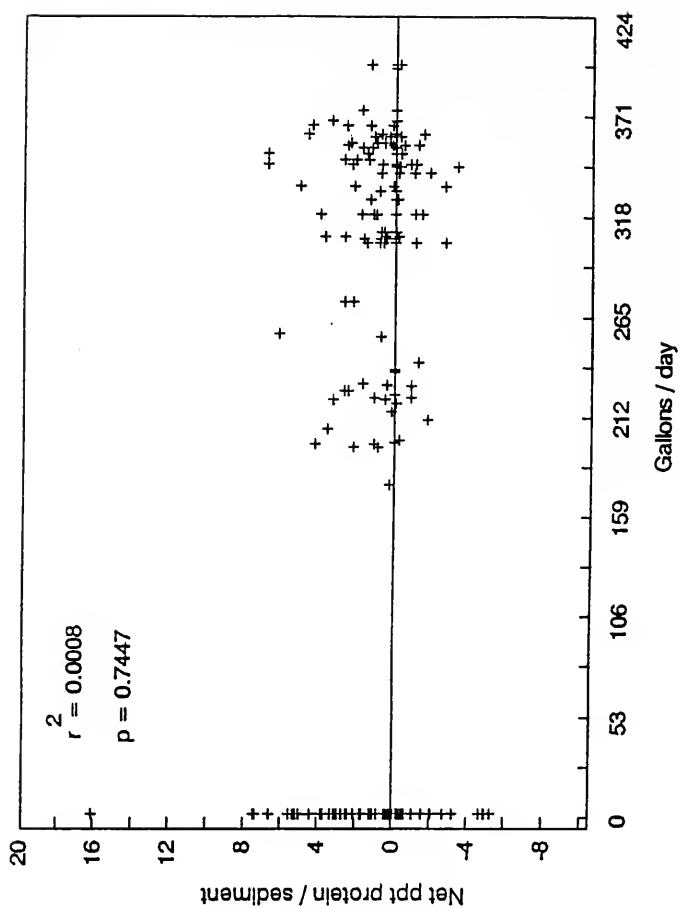


Figure 23. Regression of net sediment protein concentration (ppt protein/sediment) and mean daily water exchange (gallons/day).

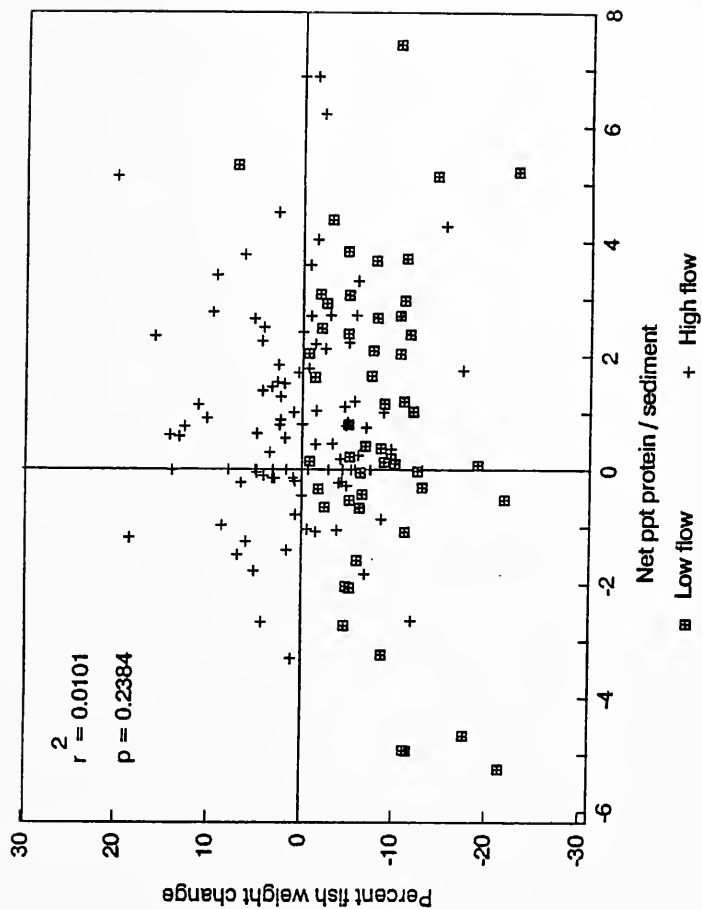


Figure 24. Regression of percent fish weight change and net sediment protein concentration (ppt protein/sediment).

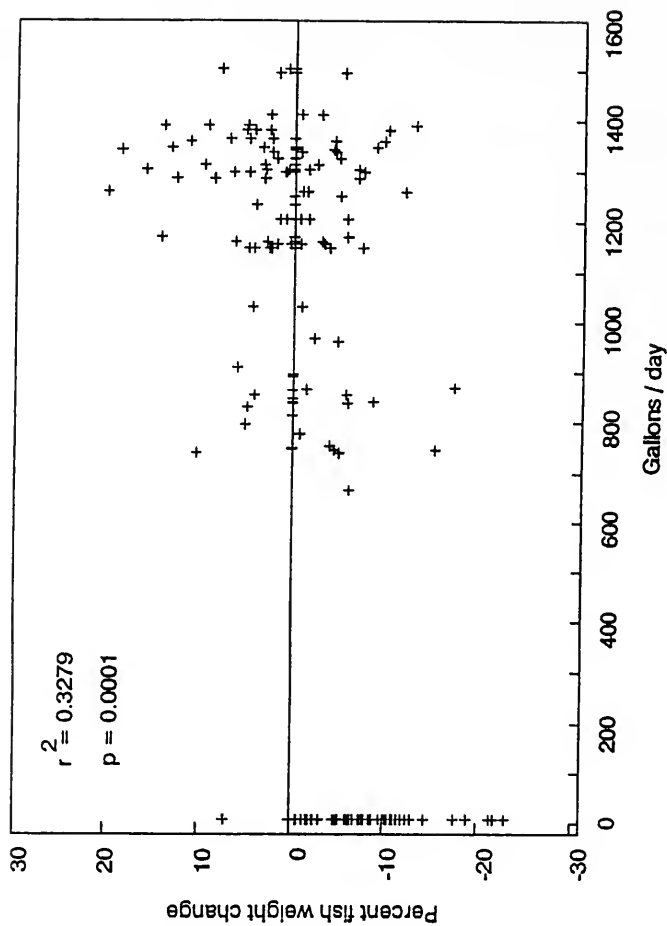


Figure 25. Regression of percent fish weight change and mean daily water exchange (gallons/day).



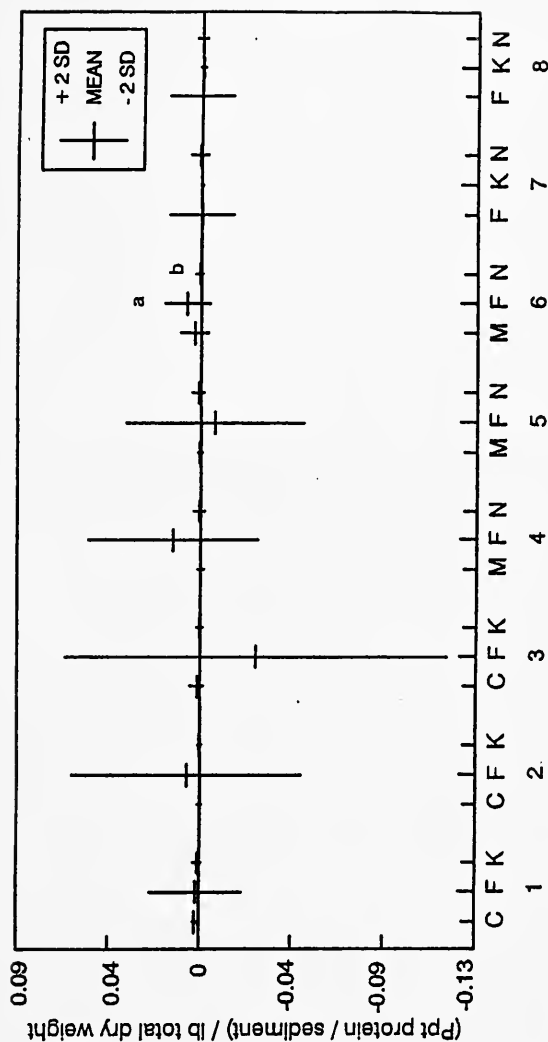


Figure 26. Net sediment protein concentration (ppt protein/ sediment) per pound dry tissue weight for each treatment in the feeding trials. Values differed significantly only in trial six ( $P < 0.05$ ). Unmarked columns within a trial are not significantly different. C - thick-shell mussel; F - fish held alone; K - fish held with thick-shell mussels; M - thin-shell mussel; N - fish held with thin-shell mussels. Numbers refer to trials.

Looking at all trials, there was no significant difference in net sediment protein produced per pound dry tissue in different mussel treatments and no significant relationship between these two variables ( $r^2 = 0.001$ ). In trial six the net protein/lb tissue was significantly greater in the fish treatment than in the mussel-fish treatment. In general, the mussel-fish treatments produced higher gross (and net) sediment protein concentrations, but the increase was apparently due to the increased body tissue mass in the tank rather than an enhanced protein producing property of the mussels themselves through the production of pseudofeces.

Sediment weight. The total amount of dry sediment collected from the tanks at the end of the trials varied significantly between treatments in six of the eight trials. In the low flow trials the tanks containing mussels had more sediment than tanks without mussels, significantly more in trials two and three. In trials four, five and six, tanks containing fish had significantly less sediment in them than blank or mussel treatment tanks. In trial eight the fish treatment tanks contained significantly more sediment than the thick-shell mussel-fish treatment tanks (Figure 27).

Water Quality. Dissolved oxygen did not differ between treatments within a trial (Figure 28). Turbidity varied significantly between treatments in trials two and three where flow (turnover) was low (Figure 29). In trials two and three, mean turbidities for the blank and fish treatments were significantly higher than the mussel

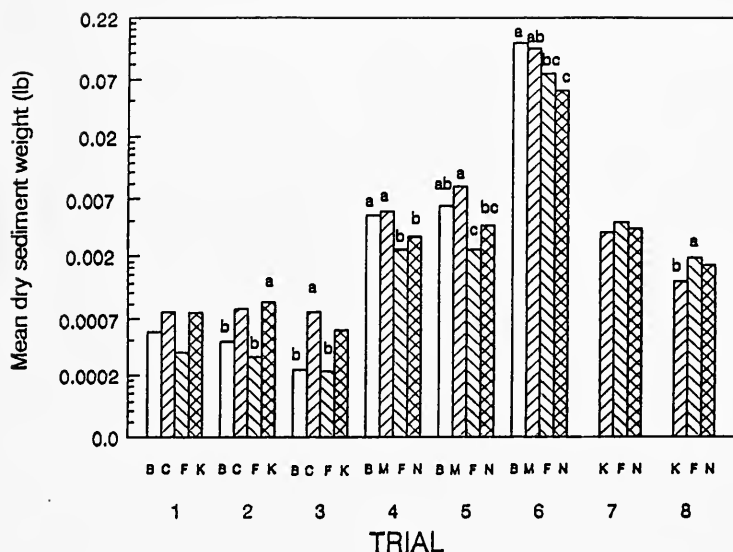


Figure 27. Mean dry sediment weight collected from each treatment in the feeding trials. Significant differences within a trial are indicated by different letters ( $P < 0.05$ ). Unmarked columns within a trial are not significantly different. B - blank; C - thick-shell mussel; F - fish held alone; K - fish held with thick-shell mussels; M - thin-shell mussel; N - fish held with thin-shell mussels. Numbers refer to trials.

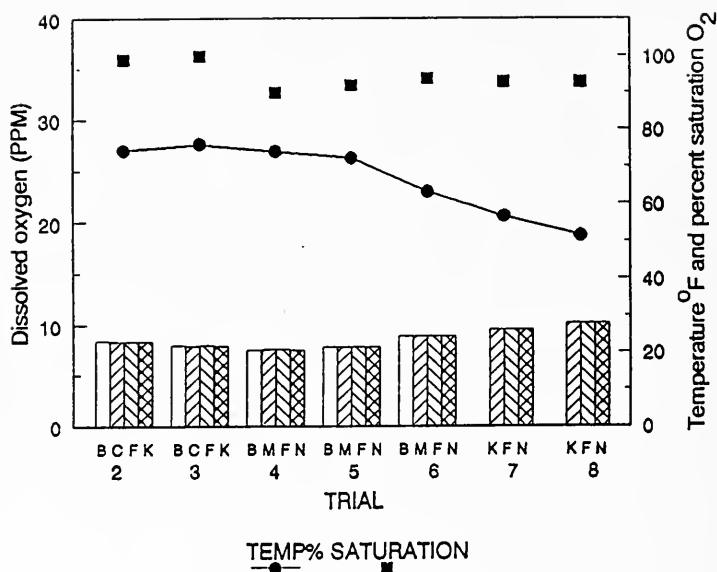


Figure 28. Concentration (ppm) and percent saturation of dissolved oxygen for each treatment in the feeding trials. No significant differences between treatments; no data were collected for trial one. B - blank; C - thick-shell mussel; F - fish held alone; K - fish held with thick-shell mussels; M - thin-shell mussel; N - fish held with thin-shell mussels. Numbers refer to trials.

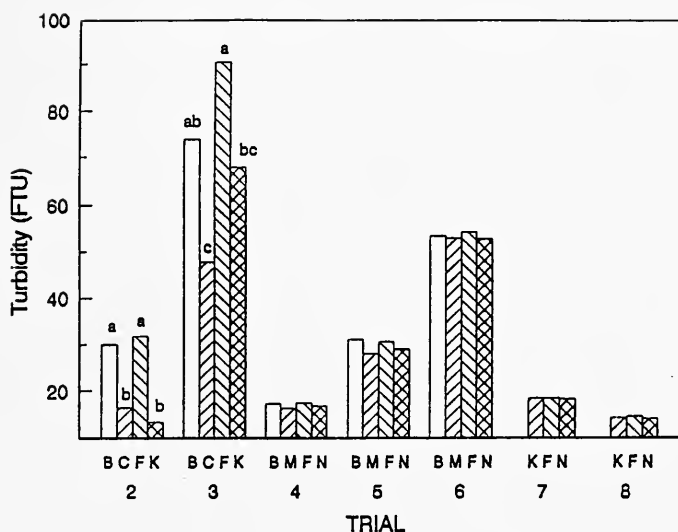


Figure 29. Mean turbidity in formazin turbidity units (FTU) for each treatment in the feeding trials. Significant differences within a trial are indicated by different letters ( $P < 0.05$ ); unmarked columns are not significantly different. No data were collected for trial one. B - blank; C - thick-shell mussel; F - fish held alone; K - fish held with thick-shell mussels; M - thin-shell mussel; N - fish held with thin-shell mussels. Numbers refer to trials.

and mussel-fish turbidities. This same pattern of turbidity differences between treatments continued through the other trials but the differences were not significant.

Figure 30 illustrates mean un-ionized ammonia concentrations, corrected for pH and temperature. These values did not vary significantly among treatments except in trial four in which mean un-ionized ammonia concentration in the mussel and mussel-fish treatments was significantly higher than that of the blank and fish treatments.

Un-ionized ammonia concentrations greater than 0.125 ppm causes reduced growth in channel catfish (Robinette 1976, Stickney 1979) and levels as low as 0.0125 ppm may adversely affect the growth of trout (Piper et al. 1986). The concentration of un-ionized ammonia exceeded 0.125 ppm in trials two, three and four and may have affected the weight change of fish in trial four. During trial four, mean ammonia concentration was lower in tanks where fish were held alone. Fish in this treatment did not lose as much weight as fish held with mussels though the difference was not significant. Elevated ammonia readings were also recorded in trials two and three; the ammonia levels in trial three were significantly higher than in trials five through eight which were the high flow trials. In trials two and three there was no significant difference in un-ionized ammonia concentration between treatments and fish held alone lost more weight than fish held with mussels.

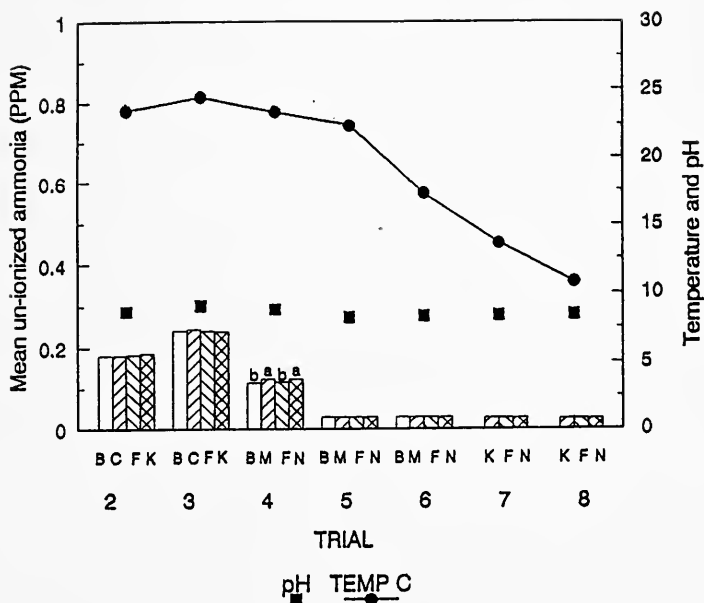


Figure 30. Mean un-ionized ammonia concentrations for each treatment in the feeding trials. Values are temperature and pH corrected. Significant differences within a trial are indicated by different letters ( $P < 0.05$ ). Unmarked columns are not significantly different. No data were collected for trial one. B - blank; C - thick-shell mussel; F - fish held alone; K - fish held with thick-shell mussels; M - thin-shell mussel; N - fish held with thin-shell mussels. Numbers refer to trials.

Mussel weights. We weighed the mussels before and after each trial, recorded the difference in weight and calculated a percent weight change for each mussel. These data are somewhat dubious though because mussels retain various volumes of water in their mantle cavity depending upon variables such as how the mussel was handled, rate of valve closure and reproductive state. Recall from Appendix F that water in the mantle cavity ranged from 16 percent body weight in thick-shell mussels to 50 percent in thin-shell mussels. The variation in how much of this water is expelled as the valves close causes difficulty in measuring mussel weights even when the mussels are weighed in water. Mussel weight change is briefly discussed below but one must bear in mind that weighing error may be significant and is difficult to avoid even with careful handling.

Mussel weight change and percent weight change were significantly different between treatments only in trials five and seven. Arcsin-transformation of percent weight change produced the same results as percent weight change. In trial five mussels held with fish gained 3.82 percent body weight (0.0083 lb) while mussels held alone lost 1.33 percent body weight (0.0069 lb). In trial seven the thin-shell mussels held with fish lost 1.98 percent body weight (0.0048 lb) and thick-shell mussels held with fish gained 0.23 percent body weight (0.0017 lb). There were no significant differences in mussel weight change in the other trials and change in mussel weight showed no consistent relationship with change in fish weight.

Table 9 lists correlation coefficients for several biological and environmental parameters with percent fish weight change. All factors were considered separately for correlation and together in a stepwise multiple regression model.



Table 9. Correlation and regression coefficients of biological and environmental variables with percent change in fish weight. Sample sizes differ due to uncollected data or absence of mussels in treatments.

Parameter	r	r <sup>2</sup>	N
Biological			
Sediment Protein	0.4344	0.1887	155
Net Sediment Protein	0.1006 <sup>a</sup>	0.0101 <sup>a</sup>	139
Dry Mussel Weight	-0.2153	0.0463	87
Dry Fish Weight	0.5159	0.2661	155
Mussel Weight Change	0.0248 <sup>a</sup>	0.0006 <sup>a</sup>	87
Environmental			
Liters/day water	0.5726	0.3279	155
Temperature	-0.3299	0.1088	155
Dissolved Oxygen	0.2046	0.0419	155
pH	-0.5233	0.2738	139
Unionized Ammonia	-0.5880	0.3457	139
Turbidity	-0.1476 <sup>a</sup>	0.0218 <sup>a</sup>	134
Sediment weight	0.4011	0.1609	155

(a) - No significant correlation ( $P > 0.05$ ). Unmarked coefficients are significant.

Net sediment protein = total sediment protein - mean sediment protein of the blank treatment in a given trial. If no blank was present (trials 7 and 8) the mean sediment protein of the fish treatment in that trial was used.

Sediment protein, sediment weight, dry weight of fish, gallons per day water exchange and concentration of dissolved oxygen exhibited significant positive relationships with percent fish weight change. Dry weight of mussels, pH, un-ionized ammonia concentration and temperature exhibited significant negative relationships with percent fish weight change.

The stepwise regression model indicated that dry weight of fish, gallons/day water exchange, temperature and sediment weight accounted for 59.23 percent of the variance in fish weight change for all tanks which contained fish (fish, and mussel-fish treatments). Dry weight of fish, sediment protein concentration and net sediment protein concentration accounted for 40.00 percent of the variance of fish weight change in the mussel-fish treatments. Heavier fish lost less weight than smaller fish. Dry weight of mussels was not a significant effect in the multiple regression analysis.

Temperature and pH interact with ammonia and affect the percent of ammonia which occurs in the un-ionized (toxic) form. Separate analysis of the high and low flow trials indicated un-ionized ammonia concentration was negatively correlated with percent fish weight change in the low flow trials. In the low flow trials warm temperatures and high pH together with low daily water exchange may have interacted with high total ammonia concentrations and adversely affected the weight change of fish in these trials. Regression analysis of the low flow trials indicated that temperature, pH, and un-ionized ammonia, and dissolved oxygen concentrations were colinear and could not be analyzed

as separate effects.

In the high flow trials water in the tanks was exchanged at a mean rate of three times per hour, a rate great enough to flush out un-ionized ammonia before it accumulated to toxic levels. The apparent positive relationship of percent fish weight change and un-ionized ammonia concentration may be a result of falling temperatures accompanied by lower concentrations of un-ionized ammonia and decreasing percent fish weight change.

## FEEDING TRIALS DISCUSSION

There was little evidence from the feeding trials to indicate that mussel feces are beneficial as forage for fathead minnows. Fish held with mussels exhibited less weight loss than fish held alone in three of the eight trials but never gained more weight than fish held alone. Significant differences existed between treatments in trials three and five. In trial three fish held with mussels lost less weight than fish held alone. In trial five fish held with mussels lost weight while fish held alone gained weight. There were several factors interacting within the series of trials which apparently influenced fish weight change to various degrees over the course of the tests.

Fish Held Alone. Weight change of fish held alone appeared to reflect changes in flow and temperature. The following discussion refers to Figure 31 which illustrates fish weight change, mean temperature, mean flow and mean mussel weight per fish in each trial.

In trial one mean temperature was 64.4 °F, this increased to the high 60's for trials two through five, then dropped to 62.6 °F in trial six, 55.4 °F in trial seven to a low of 50.0 °F in trial eight. In trials one through three water exchange was two gallons per day, in trial four water exchange increased to about 220 gallons per day

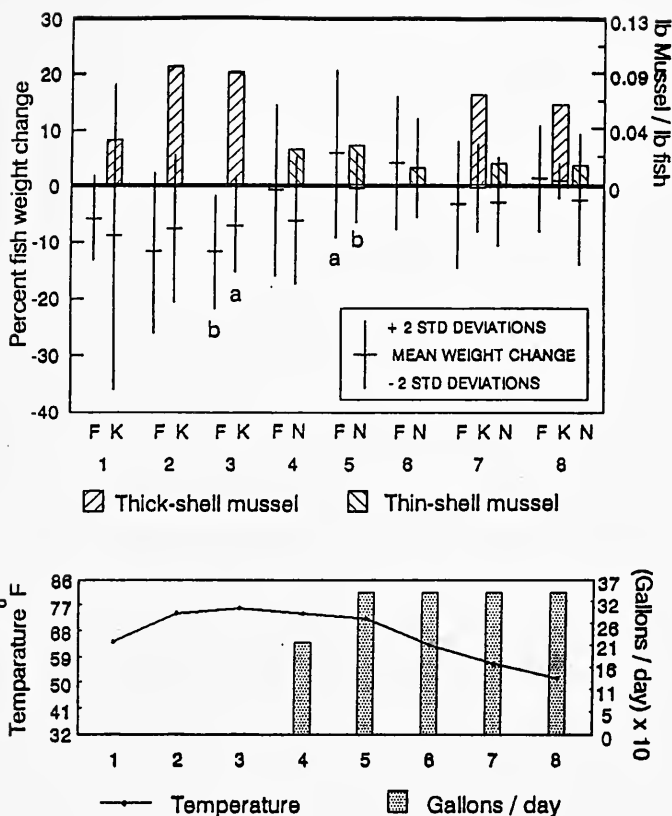


Figure 31. Percent fish weight change in the feeding trials compared with mussel-to-fish weight ratio, temperature and daily water exchange (gallons/day). Significant differences in percent fish weight change between treatments within a trial are indicated by different letters ( $P < 0.05$ ), unmarked treatments are not significantly different. B - blank; C - thick-shell mussel; F - fish held alone; K - fish held with thick-shell mussels; M - thin-shell mussel; N - fish held with thin-shell mussels. Numbers refer to trials.

and reached a maximum stable value for the remainder of the trials at 339 gallons per day. The low flow was intended to limit external sources of forage so that the minnows would have relatively little to consume except for mussel feces.

Weight change of fish held alone appeared to reflect changes in flow and temperature, more so than the weight change of fish held with mussels. Fish held alone lost five percent body weight in trial one where flow was two gallons per day and temperature was 64.4 °F. In the next two trials flow was maintained at two gallons per day, but temperature increased to 73.4 and 75.2 °F. In these two trials fish held alone lost about eleven percent body weight, perhaps in response to increased metabolic demand created by high temperature and the extremely limited forage present in the low volume of daily water exchange. Temperature remained fairly steady through trial four at 73.4 °F but mean flow was increased to about 220 gallons per day. This increased the forage available to the fish which may be reflected in the low percent weight loss of the fish held alone in trial four. This pattern continued into trial five where temperature remained at 71.6 °F and flow increased to 339 gallons per day. In trial five fish held alone gained eight percent body weight, though the sample size was only seven due to escapes.

In trial six mean temperature decreased to 62.6 °F. Mean percent weight change decreased slightly as well, though the decrease in weight change cannot be attributed to temperature alone since there were two fish held per tank in trial six.

In trial seven, temperature continued to decrease to a mean of 55.4 °F. Fish held alone lost three percent body weight which appeared to reflect the decrease in temperature. In trial eight, mean temperature was 50.0 °F; fish held alone in this trial gained two percent body weight. Two factors might have affected the results of trials seven and eight: temperature fluctuation and reduction of treatment sample size due to mortalities. The temperature in trial seven dropped from 64.4 to 46.4 °F over a period of four days in the middle of the trial, then rose again to 55.4 °F by the end of the trial. Though the mean temperature was low in trial eight 50.0 °F, temperature remained fairly stable ranging from 48.2 to 53.6 °F over the course of the trial. Four fish were lost to mortality in the fish alone treatment in trial eight; the mortalities were not size selective. The low sample size and moderate temperature fluctuation in trial eight may have resulted in the apparent increase in mean weight of fish held alone, but change in fish weight was not significantly different between trials seven and eight.

Fish Held With Mussels. The mean weight change of fish held with mussels appeared to vary with temperature and flow but was also affected by the presence of mussels in the tank. In trial one, fish were held with a variety of sizes of mapleleaf mussels with mussel tissue/fish ratios ranging from 0.013 lb mussel/lb fish to 0.138 lb mussel/lb fish, this wide range of mussel sizes may be reflected in the large standard deviation of the mean weight change for fish held with mussels in trial one. In trials two, three and four weight loss of fish held with mussels was about seven percent body weight, even

though flow increased 100-fold and the weight of mussel per fish decreased by about 60 percent in trial four.

In trials two and three fish held with mussels lost less weight than fish held alone. In these trials forage was very limited and temperature was 73.4 to 75.2 °F. In trial four, where flow (and forage) increased, weight change of fish held with mussels was less than fish held alone. Perhaps at the very low forage levels in trials two and three the fish were able to take advantage of mussel feces, since mussels can filter and coalesce particles which would otherwise be too small for the fish to consume. But when other sources of forage were available the mussels may have competed to a degree with the fish.

The results of trial five are similar to trial four. Fish alone gained weight while fish held with mussels lost weight.

The results of trials six are difficult to interpret since there were two fish per tank which decreased the net weight of mussel per fish and temperature decreased. Fish held alone gained slightly less weight than did fish in trial five, while fish held with mussels gained the greatest mean weight of any mussel-fish treatment.

Mean turbidity in trial six was highest among the high flow trials. Mussels do not produce pseudofeces until suspended particle concentrations surpass what is required for maintenance (Winter 1978). Perhaps the higher turbidity present in trial six allowed the mussels



to produce pseudofeces, and the fish held with the mussels were able to consume these materials and thus exhibit less weight loss or greater weight gain than fish held with mussels in the previous trials. The filtering action of the mussels in the small tanks may still have competed with the fish for small food items resulting in less weight increase than that exhibited by fish held alone.

It is difficult to assess what might have occurred in trials seven and eight. Perhaps the presence of mussels in the tank maintained a constant source of forage and thus allowed fish held with thick-shell mussels to maintain their weight, while fish held alone lost weight. The fish in the thick-shell mussel treatment were held with 0.070 lb mussel per lb fish, while the thin-shell treatment had only about 0.015 lb mussel per lb fish. It is likely that proportionately more feces and pseudofeces were present in the tanks with the higher mussel-to-fish weight ratio than in tanks with lower ratios. This difference in weight of mussel per weight of fish is probably responsible for the difference in fish weight change between the two treatments. Again in trial eight temperature did not fluctuate as widely as in the previous trial; all three treatments lost less weight or gained more weight than the same treatments in trial seven. As in trial seven, fish held with thin-shell mussels in trial eight lost more weight than fish with thick-shell mussels perhaps in response to the increased weight ratio of mussel/fish in the thick-shell mussel treatment tanks.

The general lack of significant results in the feeding study is probably due to several factors. First, fish probably do not benefit from the consumption of mussel feces under conditions normally found in the wild. Fish may consume this material but significant nutritional benefit from the forage was not indicated by this study. Although the water was strained through fine netting there were zooplankton and occasional insect nymphs and larvae present in the tanks. The chance appearance and consumption of these forage items in a tank could decrease the weight loss of the fish in that tank in comparison to tanks in which the fish did not obtain extra forage. Second, the slight weight changes among treatments may have been too small to register a significant difference with the sample sizes we were using. The results of the feces feeding trials suggest that mussel feces and pseudofeces are not a valuable forage for fathead minnows.

## SUMMARY AND CONCLUSIONS

This study reviewed the literature on interactions between fish and mussels and used three approaches (substrate preference tests, predator-prey trials, and feeding trials) in a laboratory setting to examine whether one forage fish (fathead minnow) and five common sport fishes (channel catfish, largemouth bass, bluegill, white crappie, and sauger) were more attracted to mussel beds than to other types of substrate, and if they were, to examine the basis for that attraction. The design and results of the laboratory experiments are summarized below. We attempted to verify the laboratory results in the field, using: (1) substrate preference tests conducted in raceways in Quiver Creek, near Havana, Illinois, and (2) fish population sampling in areas with and without mussel beds in the Mississippi River 50 miles upstream from St. Louis, Missouri. Repeated floods interrupted the raceway tests, which are not discussed further. Results of the fish population sampling were inconclusive because of sampling limitations (see Appendix G), but an improved sampling design is included in the recommendations at the end of this section. We fortuitously observed two phenomena that were incidental to the objectives of this research and that consequently are described in appendices: (1) the mantle flap display in the yellow sand shell, Lampsilis teres, (Appendix A)--the first (to our knowledge) videotaping and description of the way this mussel attracts its fish hosts, and (2) the remarkable survival of freezing by seven species of mussels (Appendix D). Information from the literature search that should interest other researchers and managers is summarized in three tables: Table 1 lists the species of fish that reportedly associate with mussel beds, together with their food habits and status as

glochidial hosts; Table 2 lists invertebrates found in association with mussels; and Appendix C the filtration rates of some marine and freshwater bivalves, including the introduced Asiatic clam (Corbicula fluminea) and zebra mussel (Dreissena polymorpha).

### Laboratory Studies

Substrate preference trials determined whether the fish spent more time over mussels than over other types of substrate (cobble and sand) and tested two hypotheses: (1) fish are attracted to the algae and small invertebrates attached to the mussel shells (the forage hypothesis), and (2) fish are attracted by the physical structure provided by the mussel shells (the structure hypothesis). The mussels were partially buried in sand in a natural position, with their pseudosiphons and the posterior portions of their shells above the sand surface. The cobble were approximately the same size as the mussels and similarly placed in sand. Individual sport fish and individuals and schools of fathead minnows were given a choice of two substrates at a time in laboratory tanks.

The forage hypothesis was tested by offering a choice between substrates colonized with attached algae and invertebrates and uncolonized substrates without attached organisms. The colonized substrates were cobble and mussels from streams. Uncolonized substrates were mussels whose shells were scrubbed clean and cobble obtained from roadsides or a quarry. The structure hypothesis was

tested by giving fish choices between bare sand and uncolonized cobbles or mussels. To check whether the shells alone were the attraction, rather than the living mussels, three species of fish (bluegill, white crappie, and juvenile channel catfish) were given a choice of empty mussel shells, as well as cobbles and live mussels. Empty shells were stored dry until placed in the preference tanks, and consequently there were no living organisms attached to them.

The predator-prey trials measured the rate at which sauger captured fathead minnows over bare sand versus uncolonized cobbles. In each of six trials five fathead minnows were introduced on two consecutive days to a substrate preference tank containing a single sauger.

The feeding studies stemmed from an observation during the preference trials of fathead minnows consuming mussel feces and a second observation of fish in the holding tanks eagerly consuming packets of glochidial larvae released by mussels. In the feeding trials, fish were held in aquaria alone or with mussels. The only food source provided during the eight two-week trials was from creek water continuously pumped into the aquaria. In every trial but one, the creek water was strained through a 0.01-inch mesh screen to remove most vegetation and invertebrates that were large enough for the fish to consume directly. The screens did not remove small particles that mussels are capable of filtering from the water. Thus the feces from the mussels were the major source of food, and the utilization and nutritional value could be judged by comparing the weight change of fish held with mussels to fish

held alone. In addition, the protein content of glochidial packets was measured to determine their potential nutritional value to fish.

Substrate preference. The five species of fishes preferred uncolonized, live mussels in 17 of 22 tests and uncolonized cobbles in only four of 22 tests. Because of time constraints, only one preference test for empty, uncolonized mussel shells was run with each of three fish species. Bluegill and channel catfish preferred empty mussel shells while white crappie avoided them; only the avoidance was statistically significant at  $P < 0.05$ . The greater preference for live mussels over rocks and empty mussel shells indicates that the fish are not merely selecting bottom substrate because of the structure it provides. The preference also cannot be explained by the presence of small invertebrates or algae on the mussel shells, which had been scrubbed.

Forage preference. Fish preferred colonized mussels in 19 of 22 tests (86%); colonized cobbles seven out of ten times (70%); and, as mentioned above, uncolonized cobbles only four out of 22 times (18%). The greater preference for colonized cobbles in comparison to uncolonized cobbles supports the forage hypothesis, as does our observation that the fish eagerly picked at the invertebrates and filamentous algae on the colonized substrates. However, there must be some attraction associated with the live mussels themselves rather than the forage attached to them, because there were no statistically significant differences ( $P > 0.05$ ) in preference among mussels with cleaned shells and mussels or cobbles with attached algae and invertebrates. Thus both

the substrate and forage tests consistently indicate some attractant associated with live mussels; or conversely, some repellent feature of uncolonized cobbles and uncolonized, empty shells. A common feature of the latter two substrates, besides having no attached live macroinvertebrates or filamentous algae, is that they were stored dry. All the other substrates were obtained from rivers and kept in water, so that their attached colonists remained alive. Some living algae and bacteria could have remained in the crevices near the umbones and hinge of even the scrubbed mussels. Marzolf (1966) noted that the amphipod Pontoporeia affinis preferred substrates whose surfaces had been "conditioned" by accumulated organic matter or bacteria, and it is possible that fish respond the same way. The possibility that mussels or their colonists release odors that attract fish was not investigated, but should be the subject of additional research. Although at least two of the 14 mussel species we used are known to employ visual lures (modified mantle flaps or glochidial packets that mimic minnows, worms, or other forage) to attract their fish hosts, none of the individuals were observed displaying during the preference trials.

Predator-prey trials. The minnows were twelve times more vulnerable to sauger when the minnows were over bare substrate than when over cobbles, and consequently they spent twice as much time over the cobbles. Sauger spent an average of 3.8 minutes per minnow capture over sand but required 11 minutes to capture a minnow over the cobble. The minnows presumably would use mussels similarly as a refuge from predation, although time did not permit testing that hypothesis in additional predation trials.

Feces as forage. Fathead minnows did not appear to benefit from the consumption of mussel feces and pseudofeces: in five of the eight feeding trials fish held alone exhibited less weight loss or greater weight gain than fish held with mussels.

Nutritional value of glochidial packets. Though mussel feces may not be nutritious forage for fish, the glochidial packets are relatively high in protein. The protein content of aborted glochidial packets from two mapleleaf (Quadrula quadrula) and two pocketbook mussels (Lampsilis cardium) ranged from 55% to 77% on a dry weight basis and the embryonic larvae in the packet had scarcely any shell material. The protein content dropped to 20% in mature packets from a yellow sandshell mussel (Lampsilis cardium), because glochidial shell material comprised a greater portion of the total mass.

#### Importance of Mussel Beds to Fishes

This laboratory study demonstrated that five common sport fishes (channel catfish, largemouth bass, bluegill, white crappie, and sauger) and one forage fish (fathead minnow) were more attracted to mussel beds than to other types of substrate. The results suggest that mussels serve as both direct and indirect sources of forage for game fish. The glochidial packets released by the mussels could provide a seasonally abundant food reward for the fishes that disperse the larval mussels. Young game fish probably consume the invertebrates that colonize mussel



shells, or the link may be from invertebrates to small fish, such as fathead minnows, to the piscivores. Sephton et al. (1980) found higher densities of invertebrates associated with mussel beds than with other substrates (the analysis included worms, insects, and snails, but excluded the mussels themselves). Another reason that small fish may concentrate in mussel beds, besides the presence of small invertebrates, is to avoid predators, as the fathead minnows did in our tests.

Small invertebrates may be more abundant in mussel beds than in other substrates because: (1) the mussels increase the surface roughness of the bottom, thereby creating vertical eddies that bring food particles in the water column into the feeding range of bottom-dwelling organisms (Holloway 1990); (2) feces and pseudofeces of the mussels provide nutritious forage (Izvekova and Lvova-Katchanova 1972); (3) the shells are a solid attachment site for eggs, pupae, and feeding nets (Anderson and Vinikour 1984); (4) the interstices among the shells provide a refuge from predators and water currents and a collecting place for food particles, including mussel feces; and (5) the movements of the mussels slowly mix the top foot or so of the substrate, probably aerating the sediment and influencing exchanges of nutrients and organic matter between sediments and the water column.

From the fishes' point of view, mussels serve another very important purpose besides providing foraging sites and refuge. Lithophilic spawners, such as walleye, apparently use both mussels and cobbles as spawning substrates (Balon 1975; Pitlo). Although none of our North American fishes are known to use mussels as brood chambers for

their eggs, several species elsewhere do, including at least 13 cyprinid species, a family with many North American representatives (Balon 1975).

Mussels can serve as solid substrate and refuges for other invertebrates and fish because most native mussels continually expose a portion of their shells above the sediment surface, rather than completely burying themselves. Although cobbles can perform the same functions, cobbles can be covered by silt or sand whereas the living mussel actively maintains its position at the sediment surface. The supply of solid substrate continually accumulates through the death of individual mussels. This is a biogenic habitat (*sensu* Woodin 1978), capable of renewing and maintaining itself despite some environmental perturbation. The solid structure provided by mussel beds is likely to be most critical as a substrate in alluvial rivers otherwise dominated by shifting deposits of sand or mud.

### Recommendations

1. Preservation and restoration of mussel beds should be regarded as an essential part of fisheries management, in view of the importance of mussel beds as a self-maintaining biogenic habitat that provides spawning sites, forage, and refuge for fish. Biogenic habitats generally cost much less to maintain in the long run than artificial substrates introduced by man.

2. The value of mussel beds to fisheries has much to do with the behavior and shell morphology of the native mussel species, therefore preservation and recovery of native mussels should be part of fisheries management. Research is needed to assess the impacts of introduced species on native mussels and to develop strategies to minimize damage and prevent other invasions. Introduced species, such as the zebra mussel, may displace the native mussels without replacing their services to other organisms, including fish. If the zebra mussel overgrows native mussels, it will interfere with their feeding and their display and release of glochidial packets and change the size and shape of the interstices available to other invertebrates and small fishes; and it may filter sufficient sperm from the water to reduce the fertilization rate of native mussels.
3. Reviews of permits for discharges or developments and assessments of damages following spills should include effects on mussels, which may be less resilient to stress than fish. Many native species of mussels require 5-12 years to reach sexual maturity and recruitment is very sporadic, so they usually are the last group of aquatic organisms to recolonize an area where their populations have been reduced or eliminated. Adult mussels cannot avoid toxicants or other stresses as readily as more mobile organisms can.

4. We recommend two approaches (a-b below) using field data to define the relationship between fish populations and mussel beds. Previous field studies of associations between fish and mussel beds suffer from the weakness that fish collections were not made over areas without beds, because the purpose of the collections was only to determine what fishes were serving as hosts for mussels. Those studies therefore are not appropriate for determining whether fish preferentially congregate over mussel beds, although our laboratory study and others provide several reasons why they might do so. Fish use of mussel beds may be highly seasonal, so field sampling should include the spring spawning season for fishes such as walleye that are lithophilic spawners, and a period including spring through summer when various species of mussels are attracting fish hosts and releasing glochidia. Fish sampling should target, or at least include fish hosts of mussels known to occur in the beds.

(a) Correlation analysis using existing data sets. The association between fish populations and mussel beds should be investigated using existing fish data sets and correlation or regression analyses. The Illinois Department of Conservation has a long-term data set on fish populations. The proximity of these stations to known mussel beds will need to be quantified, and it may be necessary to update mussel surveys in some areas.

(b) Special surveys and field experiments. Quantitative sampling of both fish and mussels should be conducted in areas where there are mussel beds and other areas where the water quality and habitat are similar, but there are few mussels. A survey approach, with a large number of replicate samples, could be adopted, or an experimental approach, or both. Quantitative sampling techniques for streams and small rivers are fairly well developed; quantitative or semi-quantitative sampling of fish in large rivers could involve some more experimental techniques such as remote sensing using fixed arrays of sonar devices over areas with and without mussel beds, deepwater electrofishing, and benthic and midwater trawling. Construction projects where entire mussel beds have been transplanted to another area would be ideal experiments, and a mussel-fish interaction study of this type should be written into these permits. Although the disturbance associated with construction is a confounding factor at the mussel removal site, the addition of mussels to the otherwise undisturbed location provides an ideal field experiment, where before-and after- measurements can be planned. Lethal episodes (spills of nonpersistent toxicants, low dissolved oxygen levels, excessive temperatures, are to be avoided at all costs, but when they do occur such episodes provide an opportunity to evaluate the effects of mussels on fish populations. The ideal situation would be to have sampled mussels and fish fortuitously before the episode, but even without predisturbance data, it is possible to measure fish populations in association with natural recovery or a planned restocking of the mussel bed.

5. Bioenergetic and population modeling of selected native mussel populations and zebra mussels could determine whether mussels contribute significantly to the food base and would also be useful in determining effects of competitors, such as zebra mussels, on the energy balance and ultimate survival of the native mussels. Mussel population models that include natural and harvest mortality would provide a rational, quantifiable basis for regulating mussel harvests, habitat disturbance, and discharges that affect growth and recruitment. The bioenergetic and population models would also be helpful in determining where mussel relocations or restorations might best succeed.

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## APPENDICES



## Appendix A. Yellow sand shell mantle flap display

On 31 March 1990 we collected about two dozen yellow sand shell mussels (Lampsilis teres) from a mudflat in pool 26 of the Mississippi river near Alton, Illinois. The mudflat had become exposed when the pool was dewatered for construction of the new lock and dam. We collected the mussels for use in the preference study and held them in outdoor tanks.

In June 1990 the yellow sand shells began to display their mantle flaps. We first noticed the display on the afternoon of 26 June. The observations in the present study are drawn from three hours of video tape recorded 27 - 28 June and 5 - 6 July 1990 of two mussels filmed in the outdoor holding tanks.

Fuller and Bereza (1974) described the structure of the yellow sand shell mantle flap from preserved specimens and compared it to L. fasciola. In L. teres there is no anterior eyespot or posterior tail differentiation as there is in L. fasciola, L. cardium and other members of the genus. The two individuals filmed showed some variation in the margin of the mantle flap; one had a smooth mantle margin while the other was fimbriated. Fuller and Bereza do not mention this variation in the mantle margin, but do suggest that mantle characters may be a means for distinguishing between very similar species.

The flapping behavior of L. teres has not been previously described but in her thorough study of lampsilid flapping behavior Kraemer (1970) discussed the mantle structure and behavior of L. cardium, L. siliquoidea and L. reeviana brevicula. Her terminology is used to describe the flapping behavior and positioning movements of the mussels.

Like cardium the sand shells performed "preparatory " movements at the beginning of the flapping display. Over a period of about 40 to 50 minutes the mussel moves from the normal position and assumes the headstand position (Figure 32), with the foot spread out on the surface of the sediment to prop the mussel up. Flap movements begin weakly and irregularly at first, then become stronger and occur more regularly and at shorter intervals. In L. teres both marsupia always protruded between the flaps, while L. cardium displayed one or both marsupia (Kraemer 1970).

Kraemer categorized the flapping behavior of L. cardium into regular and slow flapping movements. Regular movements have high flapping frequencies of 60 or more moves per minute, slow movements occur at rates of less than 30 moves per minute. The flap movements we observed in L. teres are very similar to the slow movements Kraemer describes for L. cardium. She states:

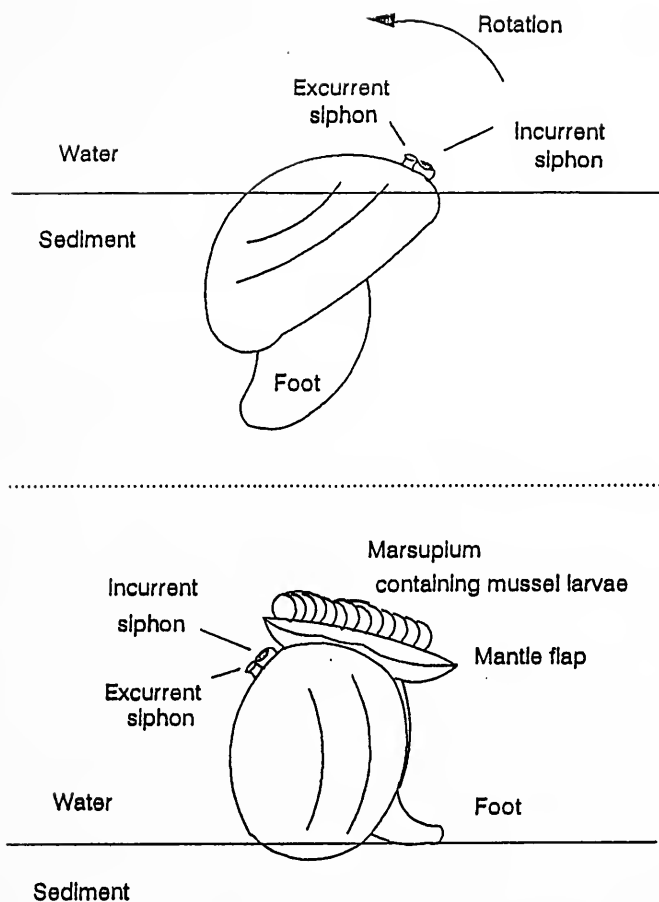


Figure 32. Repositioning movement of a yellow sandshell mussel to assume the headstand position for the mantle flap display.

"Before the slow movement starts the flaps are spread wide apart, the entire length floating out horizontally, inner sides uppermost in the water. The marsupium may not but more often does, protrude between the flaps. When the movement begins, there is a contraction at the flap base; the tails move up and touch medially; then a pulse moves from in front of the tails, which draws the eyespot ends of the flap upright, together and backward" (Kraemer 1970 p. 243).

The basic inward movement of the flap described above is the same; the recovery movement is also similar.

"In recovery, first the tails, then gradually the rest of the flaps relax and float out horizontally once more. At the end of the recovery stroke, the flaps have moved forward slightly again" (Kraemer 1970 p. 243).

The display in L. teres differs from L. cardium in that during the height of the display, the flaps are firmly pressed against the sides of the marsupia and alternately contracted anteriorly then posteriorly two or three times in unison so that both marsupia move with the flaps.

The marsupia themselves are composed of numerous glochidial conglutinates and thus appear segmented. The marsupium has a white base color with gray pigmentation distally. One individual had a single brilliant white spot dorsally in the center of each marsupium. The shaking or quivering movement of the marsupium caused by the flaps further serves to give the impression of a moving worm or insect larva.

The series of contractions - inward movement, antero-posterior contractions and relaxation of the flaps takes about two to three seconds; eleven to twelve series of movements occur in a minute.

A total of eleven yellow sand shells mussels displayed their mantle flaps over a two- to three-week period, though most of the mussels displayed only at night. This contrasts with the rapid flapping behavior of L. cardium observed by Kraemer. She reports that most of the flapping took place during the day, beginning at sunrise and ending at sunset, but the slow movements she observed took place at low light levels over long periods of time.

The earliest the two most frequently observed mussels began flapping was 08:30 and other mussels which began later, after sunset, flapped at least as late as 23:30, though the flapping behavior always ceased by morning. The mussels ceased their displays during the second week of July. Morning water temperature in the tanks averaged 73 °F and dissolved oxygen averaged 6.7 ppm. Ammonia and pH were measured weekly, pH was 8.9, mean un-ionized ammonia concentrations was 0.16 ppm.

Production of the glochidia and the flapping behavior may be energetically costly for the mussels. The marsupium of one individual comprised 44 percent of the total dry tissue weight of the mussel. The mussels which performed the display over the two- to three-week period died shortly after the display activities ceased. One possible reason for the mortalities may be that the mussels were unable to disperse the glochidia since fish were not present in the tanks. We placed two white crappies in the tank with the mussels, but the fish were more interested in nest construction than the mantle flap display.

In addition to Kraemer, other authors (Ortmann 1911, Wilson and Clark 1912, Coker et al. 1921, Howard and Anson 1922, Grier 1926, Welsh 1969) have discussed mussel mantle flap behavior and theorized on its function. Two main hypotheses surface repeatedly: attraction of a fish host and aeration of the glochidia. We feel the minnow-like mantle flap of L. cardium and the larvae- or worm-like appearance of the L. teres marsupia tend to support the host attraction hypothesis.

This highly visual mantle flap display probably helps attract sight feeding hosts. Hosts for the yellow sand shell include white crappie, largemouth bass, smallmouth bass and other centrarchids. Turbid water can interfere with fish feeding (Vinyard and O'Brien 1976) and thus may interfere with the reproduction of lampsilid mussels but the flapping behavior could attract a potential host by creating pressure waves that fish could sense with their lateral line systems.

## Appendix B. Protein content of glochidial conglomerates.

Glochidial packets from three mussel species were collected from the outdoor holding tanks at the Forbes station. Four samples, two each from mapleleaf (Quadrula quadrula) and pocketbook (Lampsilis cardium) mussels were aborted packets shed after handling and transportation. The other two samples were mature packets from recently dead yellow sand shell mussels (L. teres). One of these mussels was collected as it was dying, the other was found after it had been dead for a day, with the packets lying next to the mussel.

The protein content of the six glochidial conglomerate samples was measured using two methods: the bicinchoninic acid (BCA) microprotein analysis (Smith et al. 1985) that we used to analyze sediment protein in the feeding trials, and Kjeldahl protein analysis.

We extracted between  $1.06 \times 10^{-5}$  and  $1.52 \times 10^{-5}$  lb of conglomerate for each specimen for the BCA analysis and used two subsamples of the extracted sample for each individual. Since the quantity of the aborted packet samples was small, the mapleleaf and pocketbook packets were pooled by species for the Kjeldahl analysis. Sample size ranged from  $8.59 \times 10^{-6}$  lb for the pocketbook mussels to  $4.59 \times 10^{-4}$  lb for the yellow sand shell mussels. The protein content measured by the BCA analysis ranged from 5.05 to 14.8 percent. Crude protein measured by Kjeldahl analysis ranged from 6.94 to 77.23 percent. Table 10 presents results of

Table 10. Percent protein content of glochidial conglutinates as determined by two methods. Means of BCA analysis are given with (subsample measurements).

Species	Specimen	Kjeldahl	Analysis
			BCA
L. teres	1	21.52 21.67	12.50 a <sup>1</sup> (12.50, 12.50)
"	2	6.94	5.435 c (5.05, 5.82)
L. cardium	3	77.23 <sup>2</sup>	11.055 ab (10.73, 11.38)
"	4		14.655 a (14.48, 14.83)
Q. quadrula	5	53.48 <sup>3</sup>	8.595 bc (8.44, 8.75)
"	6		11.155 ab (9.56, 12.75)

1) Means with different letters are significantly different ( $P < 0.05$ ).

2) Specimens three and four pooled for Kjeldahl analysis.

3) Specimens five and six pooled for Kjeldahl analysis.



the two analyses.

The Kjeldahl protein analysis indicated aborted packets were higher in percent protein than mature packets. This difference may be due to the presence of shell material in the advanced conglomerates, which adds mass but not protein.

ANOVA indicated significant differences in percent protein between individuals but not between species. This suggests that there was no significant difference between mature and aborted packets, but that packets collected after sitting on the bottom of the tank for a day, (specimen two) had significantly lower protein content than fresh packets from the same species (specimen one).

The total percent protein measured by the BCA analysis tended to be less than that measured by the Kjeldahl analysis. This difference is probably due to the relatively mild extraction technique we used with the BCA method versus that used in the Kjeldahl analysis. The Kjeldahl method uses sulfuric acid to extract nitrogen from the proteins (Maynard et al. 1979); for the BCA analysis we immersed the sample in one percent solution of sodium lauryl sulfate (SDS), a detergent, at 140 °F for four hours.

It is reasonable to assume the percent crude protein values from the Kjeldahl analysis are absolute protein values, therefore by comparison, the BCA method extracted from 11 to 84 percent of the total protein present in the samples. This suggests that when used in this manner, one percent SDS is an inefficient extraction solution for

protein in glochidial conglomerates.

The results do provide basic information on the protein content of glochidial packets and indicate that aborted conglomerates contain protein comparable to that in prepared fish diets (Klar and Parker 1989, Brown and Robinson 1989, Stickney 1979). Mature conglomerates, though lower in protein, could form a seasonally abundant supplemental food source for fish, but the nutritional value of the packet appears to diminish quickly when exposed to leaching effects of water.

Appendix C. Filtration rates of some marine and freshwater bivalves.

Species	Filtration rate/10 <sup>-4</sup>		Reference
<i>Anodonta cygnea</i>	0.9-1.1	gal/h/lb <sup>a</sup>	DeBruin and Davids 1970
<i>A. cataracta</i>	0.8-5.0	gal/lb/h	Paterson and Cameron 1985
<i>Elliptio complanata</i>	28.8	gal/muss/h	Leff et al. 1990
"	0.2	gal/h/lb	"
"	1.9	gal/h/lb	Paterson 1984
<i>Dreissena polymorpha</i>	5.3-75.8	gal/clam/h	Widdows et al. 1990
"	0.2	gal/h/lb	Izvekova and Lvova-Katchanova 1972
"	132-158	gal/clam/h	Reeders and Bij de Vaate 1990
<i>Corbicula fluminea</i>	114.0	gal/clam/h	Leff et al. 1990
"	29.0	gal/clam/h	Habel 1970
"	3619	gal/clam/h	Lauritson 1986
<i>Sphaerium striatinum</i>	0.15-22.1	gal/clam/h	Hornbach et al. 1984
<i>Mytilus edulis</i>	6.98-14.5	gal/h/lb	Carver and Mallet 1990
"	8.9-39.4	gal/h/lb	Widdows et al. 1990
"	21.9	gal/h/lb <sup>b</sup>	Mohlenberg and Riisgard 1979
<i>Cardium echinatum</i>	14.2	gal/h/lb	"
<i>C. edule</i>	32.3	gal/h/lb	"
<i>Modiolus modiolus</i>	22.3	gal/h/lb	"
<i>Arctica islandica</i>	16.8	gal/h/lb	"

a) Estimated using filtration rate reported by DeBruin and Davids with dry weight conversion factor for *A. grandis* from the present study.

b) Rates calculated using equations in Mohlenberg and Riisgard based on 0.002 pound body weight.

#### Appendix D. Survival of frozen mussels.

The outdoor tanks where we held the mussels froze solid early in the winter of 1990 before we could move the mussels to Quiver Creek. Upon cleaning the tanks in the spring we were surprised to find that some of the mussels had survived. We are certain the tanks froze completely since they were on the surface of the ground, are not protected from the weather and not insulated in any way.

The surviving mussels were not of a single species or size class. (Table 11). Two floaters, a three-ridge and a white heel-splitter were quite large, shell length > 4.9 inches; and there was one young floater and one young washboard mussel. The other survivors were mature individuals of various species averaging 2.3 inches in length.

Intertidal mussels survive freezing when exposed to air at low tide in northern latitudes (Storey and Storey 1990). Freezing is an unlikely event on a large river, though temperatures in the main channel are near freezing for much of the winter (Sheehan et al. 1990). Shallow backwater areas can freeze to the bottom in a severe winter (Bodensteiner et al. 1990). Since mussels are adapted to survive long periods of near freezing temperatures in main channel habitats. It is possible that they have evolved adaptations for survival of freezing.

Table 11. Number of mortalities and survivors of mussels frozen in outdoor tanks.

Species	Mortalities	Survivors	Percent Survival
<i>Amblyema plicata</i>	8	2	20.0
<i>Anodonta grandis</i>	34	4	10.5
<i>Arcidens confragosus</i>	1	-	0.0
<i>Lampsilis teres</i>	1	1	50.0
<i>Lasmigona complanata</i>	7	1	12.5
<i>Leptodea fragilis</i>	1	-	0.0
<i>Megaloniais nervosa</i>	3	1	25.0a
<i>Obliquaria reflexa</i>	0	2	100.0
<i>Potamilus alatus</i>	1	-	0.0
<i>Quadrula quadrula</i>	20	5	20.0
<i>Q. pustulosa</i>	2	-	0.0
Total	78	16	17.02

a) Two adult and one juvenile washboard died, the survivor was a juvenile.

Appendix E. Total time (fish-minutes) spent over sand and rock in the predator-prey trials by sauger and fathead minnows.

Fish-minutes equal the number of fish present over a given substrate during a time interval multiplied by the length of the interval. Trials began with five minnows and one sauger. The adjustment factor adjusts the length of the trial to a mean duration by species for calculation of mean percent time spent over each substrate for each species.

Sauger							
Trial	Fish-minutes			Mean Total	Adjustment Factor	Adjusted Fish-minutes	
	Sand	Rock	Total			Sand	Rock
1	7	8	15	35	2.33	16	19
2	48	14	62	35	0.56	27	8
3	48	0	48	35	0.73	35	0
4	6	23	29	35	1.21	7	28
5	10	1	11	35	3.18	32	3
6	10	36	47	35	0.76	8	27

Fathead Minnow							
Trial	Fish-minutes			Mean Total	Adjustment Factor	Adjusted Fish-minutes	
	Sand	Rock	Total			Sand	Rock
1	17	28	45	72	1.60	27	45
2	130	74	204	72	0.35	46	26
3	38	45	83	72	0.87	33	39
4	3	26	29	72	2.49	8	64
5	4	11	15	72	4.81	19	53
6	8	49	57	72	1.27	10	62

Appendix F. Calculation of percent dry tissue weight from whole wet body weight for mussels used in the feeding trials.

Mean whole and partial wet and dry weights for the sample of ten three ridge (thick) and ten floater (thin) mussels. Mean shell length: three-ridge 4.49 inches, floater 3.56 inches. Length measured as maximum antero-posterior dimension of the shell. All weight measurements in pounds.

	Three ridge	Floater
Total Wet Weight	0.79	0.24
Tissue Wet Weight	0.12	0.07
Shell Wet Weight	0.55	0.04
Water Weight <sup>a</sup>	0.13	0.12
Total Dry Weight	0.55	0.04
Tissue Dry Weight	0.016	0.004
Shell Dry Weight	0.534	0.038
Water Weight <sup>b</sup>	0.24	0.20
Percent Dry Weight <sup>c</sup>	69.62	16.67
Percent Dry Tissue <sup>d</sup>	2.02 (0.6515)	1.66 (.1476)
Percent Dry Shell	67.59	15.83
Percent Moisture	30.38	83.33

a) Water contained in mantle cavity.

b) Difference between wet and dry total weights.

c) Measured as (weight)/(total wet weight).

d) Used as conversion factor for total wet weight to dry tissue weight. Standard deviation in parenthesis.

Appendix G. Methods and results of field sampling at pool 26 of the Mississippi River.

We identified three sites at the upper end of pool 26 of the Mississippi River as non-mussel, mussel sanctuary and exploited mussel bed areas. The non-mussel site was located on the west bank at river mile 240, the sanctuary site was on the east bank at river mile 238.4 and the exploited site was on the east bank at river mile 233.5. The sites were sampled from 20 through 23 August 1991.

Substrate at each site was sampled with a petite Ponar dredge and the presence or absence of mussels was confirmed using a five foot brail bar. Current velocity, temperature, dissolved oxygen, pH, conductivity and alkalinity were sampled at the surface daily at each site. Water clarity was measured with a Secchi disk.

Physico-chemical Parameters. Mean alkalinity, pH, conductivity and current velocity did not differ significantly between the three sites. Mean Secchi disc readings were significantly greater at the non-mussel site than at the sanctuary site. Mean Secchi disc reading at the exploited bed did not differ from either of the two other sites. Temperature and saturation of dissolved oxygen were significantly higher at the exploited bed than at the other two sites (Table 12). Though the differences between sites in oxygen and temperature were statistically significant, the differences may be insignificant ecologically since



Table 12. Mean physico-chemical parameters measured at a non-mussel area, a mussel sanctuary and an exploited mussel bed in pool 26 of the Mississippi river. Values of a given parameter with different letters varied significantly between sites. Unmarked values did not differ significantly.

Parameter	Site		
	Non-mussel	Mussel sanctuary	Exploited bed
Temperature °F	75.2 <sup>b</sup>	75.2 <sup>b</sup>	76.6 <sup>a</sup>
Dissolved oxygen concentration (ppm)	9.0 <sup>b</sup>	9.3 <sup>b</sup>	11.3 <sup>a</sup>
saturation (%)	106.00 <sup>b</sup>	109.75 <sup>b</sup>	132.75 <sup>a</sup>
Alkalinity (ppm).	188.1	188.1	188.1
pH	8.4	8.6	8.6
Conductivity (umho)	482.50	471.25	473.75
Current velocity (ft/sec)	1.65	1.32	2.91
Secchi disc depth (inches)	14.2 <sup>a</sup>	11.6 <sup>b</sup>	11.9 <sup>ab</sup>

dissolved oxygen was at 100 percent or greater saturation each day at all three sites and mean temperature was only 1.4 °F higher at the mussel sanctuary site.

Substrate at the non-mussel site was sand; at the other two sites the substrate was rock or firm mud colonized with mussels. Substrates retrieved with the ponar dredge from the mussel sanctuary and exploited mussel bed were heavily colonized with insect larvae tentatively identified as caddisflies (Trichoptera). During the week of sampling, adults of these insects were abundant enough along the river banks to be a nuisance.

Fish sampling. Fish were sampled using an AC electrofishing boat and large and small hoop nets in tandem sets, one tandem set at each site. About 125 yards of shoreline were electrofished at each site on all days. Electrofishing runs were seven minutes long; half the time was spent going downstream, the remaining time was used to go back upstream past the same section of shoreline. The nets were set overnight and checked each day for three days.

The species and length of each fish were recorded for each site each day. Fish were released at the site. Mean fish length and mean number of fish captured were compared between sites. Electrofishing and hoop net catches were pooled for statistical analysis.

Results. The mean daily number of fish caught did not differ significantly between sites nor was there a significant difference in

the mean number of species between the sites. The number of fish species caught over the four-day sampling period was highest over the exploited bed (N=16), lowest over the sanctuary (N=11), and moderate over the non-mussel area (N=14). Table 13 shows the species captured at each of the three sites. We have roughly categorized the species into three groups: sport fish, forage fish and "other" species. This last group contains both commercial and non-commercial species.

The mean number of fish of a given species caught daily varied significantly among sites in four species: black crappie (Pomoxis nigromaculatus), drum (Aplodinotus grunniens), common carp (Cyprinus carpio), and emerald shiner (Notropis atherinoides). Black crappie were captured only at the non-mussel site. Drum were significantly more abundant at the exploited mussel bed site than the non-mussel site. Carp were significantly more abundant at the mussel sanctuary site than at the exploited mussel bed, but the number of carp captured at the non-mussel site did not differ significantly from the number of carp caught at either of the other two sites. Significantly more emerald shiners were caught in the non-mussel area than in the sanctuary, but the number caught at the exploited site did not differ significantly from the other two sites. Mean daily catch of the other species did not differ significantly between sites (Figure 33).

Table 13. Species composition at each site in pool 26 of the Mississippi River. Presence at a location denoted by 'X'.

Group	Species	1	Site 2	3
Sport	<i>Pomoxis nigromaculatus</i>	X		
	<i>Lepomis macrochirus</i>	X	X	X
	<i>Micropterus salmoides</i>	X	X	X
	<i>Morone chrysops</i>	X	X	X
	<i>Morone mississippiensis</i>			X
	<i>Ictalurus punctatus</i>	X	X	X
	<i>Pylodictus olivarius</i>	X	X	X
Forage	<i>Dorosoma cepedianum</i>	X	X	X
	<i>Dorosoma petenense</i>	X	X	X
	<i>Notropis atherinoides</i>	X		X
	<i>Notropis blennioides</i>		X	
	<i>Hybopsis storeriana</i>			X
Other	<i>Alosa chrysochloris</i>	X		X
	<i>Aplodinotus grunniens</i>	X	X	X
	<i>Cyprinus carpio</i>	X	X	
	<i>Carpionotus carpio</i>		X	X
	<i>Lepisosteus platostomus</i>	X		X

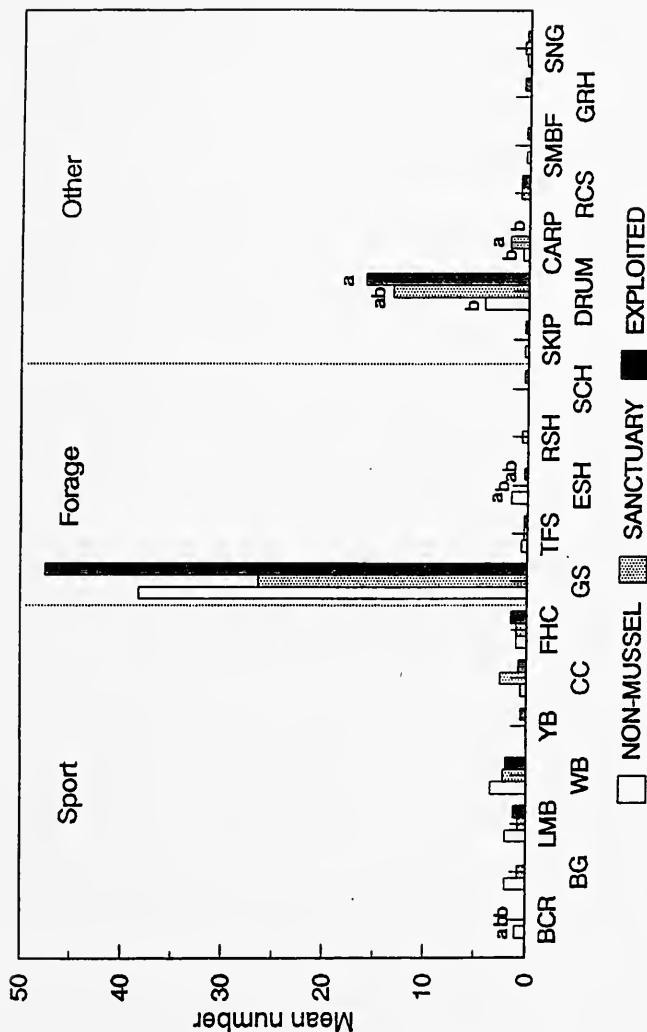


Figure 33. Mean number of each species of fish captured in pool 26 of the Mississippi in non-muskel, muskel sanctuary and exploited muskel bed areas. Columns with different letters within a species are significantly different. BCR - black crappie; BG - bluegill; LMB - largemouth bass; WB - white bass; YB - yellow bass; CC - channel catfish; FHC - flathead catfish; GS - gizzard shad; TFS - threadfin shad; ESH - emerald shiner; RSH - river shiner; SCH - silver chub; SKIP - skipjack herring; DRUM - freshwater drum; CARP - common carp; RCS - river carpsucker; SMBF - smallmouth buffalo; GRH - golden redhorse; SNG - shortnose gar.

Gizzard shad were by far the most numerous fish captured (total 449); the next most numerous species was drum (total 134), followed by white bass with a total of 31 fish caught. Total catch numbered sixteen or less for each of the other species over the four-day trial (Figure 34).

Mean length of drum was significantly greater at the non-mussel site than at the exploited bed. Mean length of flathead catfish caught over the two mussel bed sites was significantly greater than that of flathead catfish caught at the non-mussel site. Mean size of gizzard shad caught over the exploited bed was significantly smaller than the mean size of gizzard shad caught at the other two areas. No other species exhibited significant differences in length between the three sites (Figure 35). Over all species, the length-frequency distributions were similar for the three sites, though more smaller fish, predominantly gizzard shad and drum, were captured over the exploited mussel bed (Figure 36).

Discussion. Over this short trial neither the number of species caught per site nor the number of fish caught per site were significantly correlated with any of the water quality variables or substrate. Over all, there were no significant correlations between species length and any of the chemical parameters. There were a few weak but significant relationships between the number of fish of a given species caught and the chemical parameters; most occurred with species which appeared infrequently in the daily catch (Table 14).

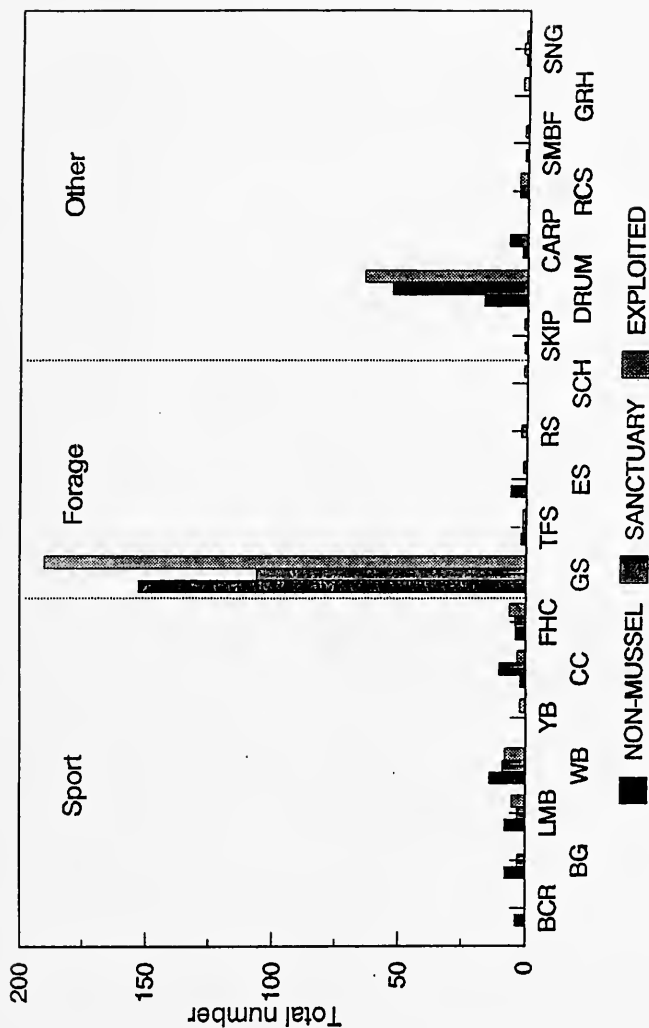


Figure 34. Total number of each species of fish captured in pool 26 of the Mississippi in non-mussel, mussel sanctuary and exploited mussel bed areas. BCR - black crappie; BG - bluegill; LMB - largemouth bass; WB - white bass; YB - yellow bass; CC - channel catfish; FHC - flathead catfish; GS - gizzard shad; TFS - threadfin shad; ESH - emerald shiner; RSH - river shiner; SCH - silver chub; SKIP - skipjack herring; DRUM - freshwater drum; CARP - common carp; RCS - river carpsucker; SMBF - smallmouth buffalo; GRH - golden redhorse; SNG - shortnose gar.

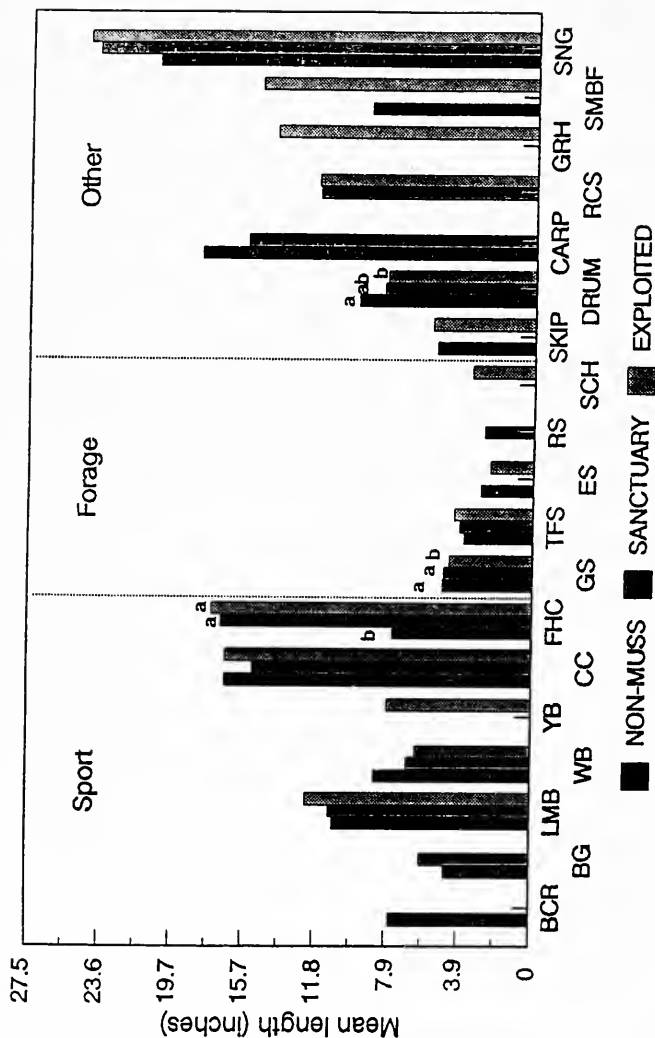


Figure 35. Mean length (inches) of each species of fish captured in pool 26 of the Mississippi in non-mussel, mussel sanctuary and exploited mussel bed areas. Columns with different letters within a species are significantly different. BCR - black crappie; BG - bluegill; LMB - largemouth bass; WB - white bass; YB - yellow bass; CC - channel catfish; FHC - flathead catfish; GS - gizzard shad; TFS - threadfin shad; ESH - emerald shiner; RSH - river shiner; SCH - silver chub; SKIP - skipjack herring; DRUM - freshwater drum; CARP - common carp; RCS - river carpsucker; SMBF - smallmouth buffalo; GRH - golden redhorse; SNG - shortnose gar.



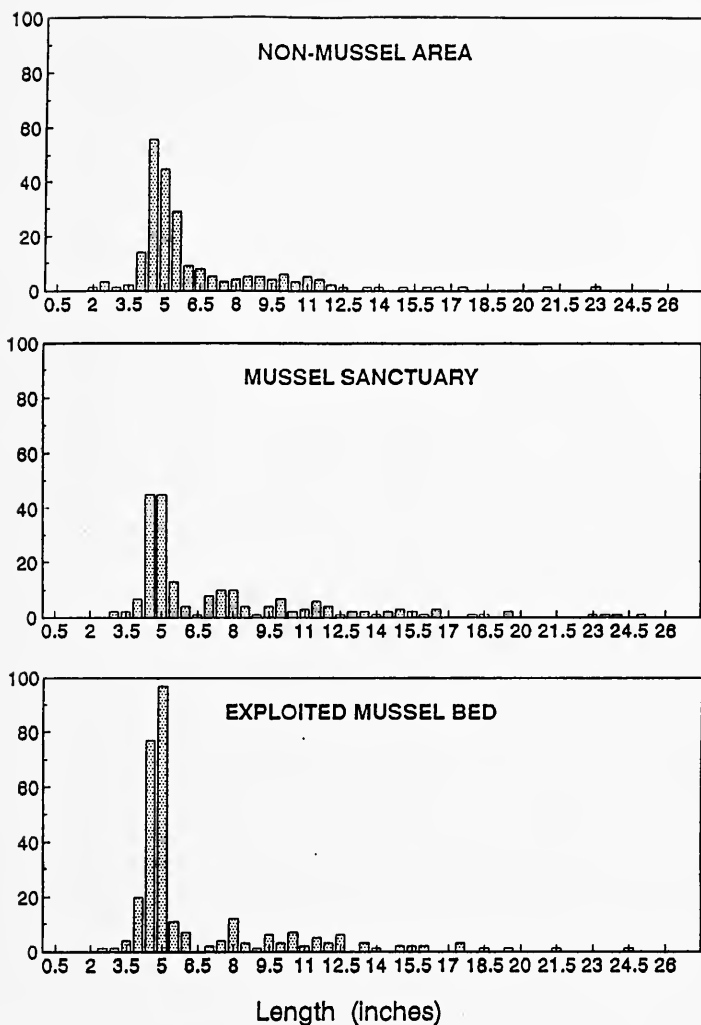


Figure 36. Combined length-frequency distributions for all species at the non-mussel, mussel sanctuary and exploited mussel bed sites in pool 26 of the Mississippi River.

Table 14. Regressions (r) between total daily number of fish captured over the four day period and physico-chemical parameters at the three sites. \* - Significant regression ( $P < 0.05$ ).

Species	Dissolved Oxygen	pH	Velocity cm/sec	Secchi cm	Temperature °C
Black crappie	-0.27	-0.36	-0.37	0.48	-0.33
Bluegill	-0.35	0.10	-0.25	0.38	-0.39
Largemouth bass	-0.34	0.02	0.32	0.54	-0.17
White bass	-0.32*	0.20	0.37	0.55	-0.17*
Yellow bass	0.78*	0.27	0.28	-0.33	0.66*
Channel catfish	-0.04	0.18	-0.18	-0.45	-0.42
Flathead catfish	0.10	0.11	0.53	0.00	0.47
Gizzard shad	-0.01	0.09	0.53	-0.18	0.14
Threadfin shad	0.09	0.15*	-0.26	0.31	-0.05
Emerald shiner	-0.21	-0.75*	-0.09	0.54	-0.19
River shiner	0.00	0.18	-0.31	-0.14	0.13
Silver chub	0.74*	0.18	-0.15	-0.14	0.45
Skipjack herring	0.47	0.18	-0.24	0.06*	0.19
Freshwater drum	0.58*	0.47	-0.18	-0.69*	0.45
Common carp	-0.34	0.26	-0.39	-0.32	-0.40
River carpsucker	0.39	0.21	0.03	-0.15	0.05
Smallmouth buffalo	-0.09	0.08	0.28	0.23	-0.04*
Golden redhorse	0.81*	0.17	-0.16	-0.21	0.66*
Shortnose gar	-0.29	0.09	0.13	0.01	-0.19

With the exception of emeral shiners, drum, carp and black crappie, these results do not indicate any striking differences in the fish populations between mussel and non-mussel sites. Part of the reason for this may be due to the relatively low catch rates of most species.

Electrofishing took 96 percent of the total catch (614 of 638 total fish). Thus the apparent lack of difference between sites is not too surprising since electrofishing was applied along the shoreline rip-rap and boulder habitat which was common to all sites. The hoop nets, which could better sample fish associated with benthic habitats, caught too few fish for statistical analysis.

Further field work should attempt to apply a wider variety of sampling strategies over a longer period of time. For example a sampling period of two to three weeks utilizing longer shocking runs, use of deep water shocking methods, and application of a variety of net types such as fyke, hoop and trap nets would increase the number of fish captured and help increase the number of species captured. Also, since different fish species may exhibit temporal variation in their distribution and may be attracted to mussel beds during periods of glochidial release, samples should be taken during the spring and summer months.

Identification of sites which are as uniform as possible from the standpoint of physico-chemical parameters in conjunction with qualification and quantification of the substrates and invertebrate forage available at each site will be essential for relating observed differences in fish populations to the presence or absence of mussel beds.





